ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL INSTITUTE OF NEUROLOGICAL AND COMMUNICATIVE DISORDERS AND STROKE

Fiscal Year 1975

Volume II

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service National Institutes of Health

Library, [illegible] Unit
National Institutes of Health
Building 10
Bethesda, Maryland 20014

ANNUAL REPORT

OF

PROGRAM ACTIVITIES

NATIONAL INSTITUTE OF NEUROLOGICAL AND COMMUNICATIVE DISORDERS AND STROKE

Fiscal Year 1975

Volume II

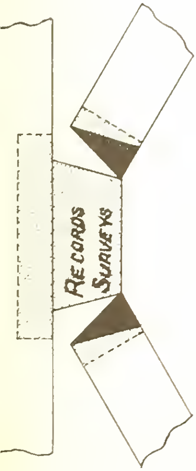
RC
346
N2776
1975
v.2

HOW TO USE
THESE SEPARATORS

Use one page for each separation.

Select appropriate tab, add further identification if desired, and cover it with scotch tape.

Cut off and discard all tabs except the one covered by tape.



TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

Annual Report

July 1, 1974 through June 30, 1975

Laboratory of Experimental Neurology, Intramural Research
National Institute of Neurological and Communicative Disorders and Stroke

William F. Caveness, M.D.

General Statement

This is a small laboratory with five permanent and one temporary position. Its productivity is dependent upon the conceptual and technical competence of each of its staff, the effective contract support for processing of anatomical specimen and supply of newborn monkeys, and the collaboration of scientists at Harvard University, and at other branches and laboratories at NIH.

This activity is a continuation of a primate laboratory established at Columbia University in 1958 as an adjunct to the Seizure Clinic for Children, Columbia Presbyterian Medical Center, both under my direction. Its initial purpose was to better understand clinical seizure patterns in infants and young children by studying the evolving seizure patterns in the developing brain of the monkey. The first eight years of this investigation is summarized in Ontogeny of Focal Seizures, chapter 19, in Basic Mechanisms of the Epilepsies, Little, Brown and Co., 1969.

In 1961, in collaboration with Brookhaven National Laboratories, a series of studies were begun on the delayed effects of x-irradiation on the cerebral cortex of the Macaca mulatta. This was prompted by the clinical observations on the wards of the Neurological Institute of New York of delayed spinal cord necrosis that followed in a matter of months the unintentional exposure of the cord during irradiation therapy for neoplasms of the nasopharynx, lung or mediastival structures. The results of the first six years of these investigations are set forth in Pathogenesis of X-irradiation Effects in the Monkey Cerebral Cortex, Brain Research, 1968, 7: Special Issue.

The Laboratory was established within the Intramural Program, NINDS, NIH April 1969 with the physical transfer of records and equipment from New York to Bethesda in March 1970.

Current Scientific Effort:

1) Experimental Focal Seizures in the Monkey

a) Ontogeny: Earlier electrographic and clinical findings, indicative of the dependence of the seizure pattern on regional organization in the central nervous system, led us to postulate that while the cerebral cortex in the newborn is able to support focal paroxysmal activity, it is dependent upon the subcortical somatotopically linked nuclear masses and circuits for the full development of the focal abnormality, its propagation to other parts of the cortex and to a common final pathway that results in the clinical seizure. In the 24 month old (pubescent) monkey, on the other hand, the propagation is under the control of corticol circuitry. To test this concept, two experimental procedures were carried out just prior to penicillin induced focal activity in the cortical motor face area. By undercutting the site of activation, the propagation was blocked in the newborn and unimpeded at 24 months of age. Interrupting the intracortical connections by circumsecting the activation site had negligible effect in the newborn, but blocked the propagation at 24 months. These findings support the concept that the effective neuronal organization for the propagation of focal paroxysmal activity shifts from subcortical to cortical with maturation. The anatomical substrate for the seizure activity in the cortex has been extensively investigated with particular attention to the developing fiber systems, as delineated by Nauta techniques, and the developing pyramidal and stellate cells as demonstrated in Golgi preparations. At birth, cortico-cortical connections are poorly developed, and cortico-subcortical circuits are well demarkated. In contrast, cortico-cortical connections are prominent at 24 months of age. This increase in cortical connectivity is reflected in the postnatal architectonic development of layers II and III, the loci of the most dramatic events of the postnatal development. These studies have been completed and the results published.

b) Subcortical Factors:

Blood Flow Studies: A logical extension of the preceding observations is a search for the subcortical circuits and nuclear masses that are involved in the propagation of the seizure activity. Previous studies by others have attempted to define "preferential" pathways by depth recording or by selective destruction of subcortical structures. For us it seemed wise to gain a global appraisal prior to the definitive study of individual elements. For this we have employed the [^{14}C] antipyrine technique for the determination of regional cerebral blood flow during selected phases in the propagation. The assumption, based on sound observations by others, is that an increase in neuronal activity will be reflected by an increase in blood flow.

With the painstaking cooperation of the Laboratory of Cerebral Metabolism, we have developed the [^{14}C] antipyrine method and added modifications that permit the sectioning of the whole head for the autoradiograms and the magnification by five of the radiograms for dosimetry. This prevents distortion in the brain sections, and the densitometric measurement of small subcortical nuclear masses respectively. In brief: The diffusable radioactive tracer, [^{14}C] antipyrine - 200 μc/kg body weight, is infused over a sixty second period during the development of a focal seizure. The monkey is then decapitated and the head immediately immersed in a Freon bath chilled to -100 C by liquid nitrogen. In short the fit is frozen in the brain. Subsequently the brain, in its case, is serially sectioned at 30 micra with a Jung K-type microtome at a cryostatically controlled temperature of -20 C. Approximately 100 sections from each brain are dried at 60 C and placed on blue sensitive X-ray film for macroautoradiographs. The densitometric readings from the structures of interest are converted to blood flow estimates by the formula of Kety.

The findings to date, from a series of 10 young adult monkeys, 8-10 Kg, indicate a consistant unilateral increase in blood flow during the propagation of focal seizures following induction in the right motor cortex. Specifically, the following structures showed a 25+% increase over their homologous structures: Right sensori-motor cortex, putamen, lateral and medial globus pallidus, substantia nigra, VA, VL, VPL, VPM, CM of thalamus, pontine nuclei, and left cerebellar anterior and paramedian lobes, dentate and interpositus nuclei. Of particular interest was the marked increase, in a roughly somatotopic distribution, in the right putamen and globus pallidus and the left cerebellar cortex. A comparable pattern of increased subcortical activity was found in pubescent monkeys, 2.5-3 Kg in weight.

Two advantages in exploring subcortical mechanisms in the newborn are being exploited. First, the level of development and integration of the cortex is such that the better developed subcortical systems are likely to have clearer expression. Secondly, the propagation of seizure activity, at this age, is protracted in time providing a better opportunity to observe the sequential involvement of neuronal aggregates. In the current newborn series, the seizure activity is being interrupted at two and five minutes following the injection of penicillin. In the first the electroencephalographic paroxysmal activity has been sporadic and limited to the right sensori-motor area and the increased blood flow has been limited to the right sensori-motor cortex and the right putamen. In some of those decapitated five minutes after the penicillin injection, the abnormal electroencephalographic activity has also been limited to the right sensori-motor area but the increased blood flow has been found in the right sensori-motor cortex, supplementary motor cortex, putamen, globus pallidus and substantia nigra. Others, decapitated at five minutes have shown well organized 2-3/sec. EEG spikes from the right sensori-motor area along with concomitant movements in the contralateral hand. Increased blood flow was found in the right sensori-motor cortex, supplementary cortex, putamen, globus pallidus, substantia nigra, subthalamic nucleus, VL, VPL, VPM and CM of the thalamus, and the left anterior lobe of the cerebellum. Note: This newborn series shows a clear cut progression in subcortical structures with the progression in electroencephalographic activity and onset of the clinical expression.

Biogenic Amines: Within the past year we have added an important parameter to the blood flow studies of seizures. With the Laboratory of Clinical Sciences, Section on Histopharmacology, we are examining the changes in cathecholamines in the areas that have shown increased blood flow. In the routine sectioning of the frozen head, when a structure of interest is exposed, first the section for the autoradiogram is taken, then a sample of the structure is obtained with an 18 gauge meedle from the block remaining in the cryostat. The material is immediately transferred to the appropriate solutions for micro-assay of dopamine and norepinephrine or choline acetyl transferase. A small numerical ticket marks the defect in the block which is then photographed. These procedures are carried out faster than they can be described with minimal elevation in cryostat temperature.

Findings to date are limited to three, 3 Kg, monkeys and only a provisional comment is justified: The two seizure monkeys show an appreciable difference when compared to a single control, and in the two seizure monkeys there are appreciable unilateral changes. Further evaluations of both the method and the findings are being carried out. The search for the role of neurotransmitters in the propagation of seizure activity will continue, with consideration for indirect (enzymes, metabolites) as well as direct evidence of their change in activity.

Electrographic Observations: A third parameter was derived from the prominence of the increased blood flow in the cerebellum during the seizure activity. The initial phase of this study involves the simultaneous recording of the electrical activity from the right and left cerebral cortex and the right and left anterior lobes of the cerebellar cortex. In six 3Kg animals we have found concomitant paroxysmal activity in the face hand area of the cerebral motor cortex and the somatotopic representation of the face in the anterior lobe of the contralateral cerebellar cortex. Further, with the advance of the seizure activity to the hand area in the cerebral cortex there is a similar advance to the hand representation in th the cerebellar cortex. These electrical phenomena are accompanied by clonic movements in the face and hand, respectively. We will add to this preparation the simultaneous recording from the dentote and interpositus nuclei and the VL nucleus of the thalamus. When this model is reliably established, we will selectively interrupt the paroxysmal activity with a cryoprobe to determine the effect of the individual elements on the propagation of the focal seizures. Note: The inability to replace a temporary position in March 1975, through administrative constraints, has markedly impeded this aspect of the studies. The temporary position was to provide technical assistance for a Visiting Scientist, highly trained in neurophysiology and recruited specifically for this work.

Significance to Bio-Medical Research and the Program of the Institute: The results from these investigations should improve the understanding of subcortical factors in focal seizures and thereby provide new approaches to medical and/or surgical therapy.

Keyword Descriptors: Focal Epilepsy - Subcortical nuclear masses - Propagation of paroxysmal activity - Regional cerebral blood flow - Neurotransmitters - Depth recording

2) Delayed Effects of Ionizing Irradiation on the Brain of the Monkey

a) Local exposure to a single dose: In the model established several years ago, the right occipital lobe in 3 Kg monkeys is exposed to 3,500 rads of orthovoltage radiation in a single dose. During the preceding and current fiscal years, three experimental procedures have been exploited to define the mechanisms operating in the long-term delayed effects in this model.

The first consisted of twelve monkeys with implanted cerebral spinal fluid catheter-reservoir systems, permitting sequential monitoring of the CSF pressure following irradiation. In eight animals there was relatively abrupt brain swelling, 18 to 35 weeks after irradiation, reflected by an increase in CSF pressure, 3-4 times baseline values, with absence of visual evoked response and behavioral blindness in four, and marked VER depression in four. Three had papilledema. Seven of these, sacrificed between 20 and 36 weeks, one hour after i.v. Evans Blue, 2 cc/Kg, showed proliferative and degenerative changes in the right occiput, deeply stained with dye. The swelling extended, beyond the irradiated and stained area, throughout the right hemisphere with distrotion in brain structures. One sacrificed at 52 weeks, after resolution of the increased pressure and return of VER, had a fibrotic, non stained occipital lesion and no brain swelling. The remaining four animals showed variations in CSF pressure, seldom exceeding twice baseline, over 52 weeks; moderate reduction in the visual evoked response; and moderately swollen brains, with Evans Blue staining of the irradiation site, at sacrifice. The significant feature was the breakdown of the blood brain barrier that accompanied the delayed radiation lesion, with the loss of serum fluid into the brain parenchyma. The impaired function was related to local destruction in the right visual cortex and widespread ipsilateral vasogenic edema and contralateral compression of the right and left visual pathways, respectively.

A second series of 8 monkeys, prepared with a subarachnoid catheter-reservoir system and irradiated as described, was utilized to determine the changes in regional cerebral blood flow by the [^{14}C] antipyrine method, during the evolution and resolution of the vasogenic edema. There was found a reduction of blood flow in white and grey matter, first regionally, then throughout the ipsilateral hemisphere and finally, throughout the brain. This was accompanied by an increase in CSF pressure, CFS lactic dehydrogenase and total protein, and clinical signs of increased intracranial pressure. With resolution of CSF pressure, there was a return to baseline of CSF chemistry and partial resolution of the other parameters. The cerebral blood flow showed a greater recovery in grey than white matter, but there remained a diffuse depression suggesting a long-term impairment in cellular metabolism and/or blood flow regulatory mechanisms.

Finally, the sequence of structural changes, proceeding from intracellular to local tissue, to remote and generalized effects, with remissions and exacerbations, strongly suggest issoimmune reactions and/or the activation of an infectious agent (slow and latent viruses). To test whether such a factor is present and transmissible, two newborn monkeys and four 100 day old monkey fetuses have received cerebral injections of material from the lesions in monkeys with postirradiation necrosis. Three monkeys have been immunosuppressed prior to and during irradiation to determine whether this will accelerate or decelerate the appearance and extent of the lesions. Electronmicroscopic evaluation of the irradiated brain and cervical lymph nodes in nine monkeys have not revealed recognizable viral particles but have shown consistantly tubuloreticular structures in endothelial cells, that have not been found in three controls. We believe these represent a cellular reaction to a virus or to an immune antigen.

The studies following single exposure of the right visual cortex to 3,500 rads of orthovoltage irradiation with attention to the resultant brain swelling have been completed and the results published. The continuation of the search for the basic mechanisms in the delayed breakdwon in neural tissue following exposure to ionizing irradiation will include the completion of the histopathology of the monkeys subjected to immunosuppression and the monkeys innoculated with radiation lesions during fetal life.

b) Whole brain exposure to fractionated doses: Following the extensive studies of the delayed proliferative and degenerative effects following a single exposure of 3,500 rads of orthovoltage radiation to a limited portion of the brain, a question that confronted us was: What, if any, of these effects would be observed if the exposure were divided and delivered to the whole brain in a manner similar to that utilized in standardized therapeutic procedures? Of particular interest was the protocol employed by the Brain Tumor Study Group, sponsored by the Cancer Institute. For their irradiation of malignant gliomas, they use a 6000 rads mid plane tumor dose over a period of 6-7 weeks with lateral parallel opposing fields. With megavoltage equipment, source axis distance of not less than 75 cm and a mid plane depth dose rate of not less than 33 rads per minute, daily increments of approximately 200 rads per day are delivered during a five day week. An exploratory effort in a monkey model is now being carried out to determine the effects on normal brain from super voltage radiation within the therapeutic range.

Twelve Macaca mulatta, 3.0 Kg in weight, received 200 rads of super voltage radiation to the whole brain, each day, five days a week. To bracket the human exposure of 6000 rads, three groups of four monkeys each received doses of 4000, 6000 and 8000 rads, respectively. The courses were four, six and eight weeks. The source was a linear accelerator with an electron beam energy of 20 MeV, that after collimation was converted to photons by tantalum foils and flattened by a lead disc to provide a nearly constant radiation field. Shielding with three to eight inches of lead provided protection except for an opening limited to the external borders of the brain, as determined by routine skull x-ray. Dosimetry by thermoluminescent dosimeters and ionizing chambers indicated a variation of less than 5% throughout phantom brains, irradiated unilaterally, from the left. The depth dose was comparable to that determined for bilateral exposure to 60 Cobalt. The depth rate at mid plane was 200 rads per minute. The source-axis distance was 150 cm. The geometry of the exposure was determined at the Armed Forces Radiobiology Research Institute and the irradiation were carried out in that facility. Four additional monkeys served as controls. One from each group has been sacrificed at 6 and 12 months; the remainder will be sacrificed at 18 and 24 months, from start of irradiation. The brains are fixed by perfusion with paraformaldehyde, embedded whole in celloidin, and cut in serial sections at a thickness of 35 micra. Every twentieth section is stained for myelin by the Loyez method and the adjacent section for nerve cells and glial nuclei with the Nissl method. An additional set, 100 sections apart, is stained with H & E and PAS, for vascular and other structures. Prior to and at each four weeks following the start of radiation, observations are conducted that include neurologic, funduscopic and electroencephalographic examinations.

The Findings to date: At six months, with 4000 rads there were no changes. With 6000 rads there had been depression in visual evoked response from the third month and at sacrifice 143 punctate lesions, one mm or less, were found, primarily in the corona radiata, centrum semiovale and internal capsule, being more frequent in the left hemisphere. These lesions were of different ages, the most recent having a necrotic core with a rim of macrophages, those older containing macrophages throughout, and the oldest showing mineral deposits. Myelin and adjacent nissl sections showed areas of palor exceeding the necrosis suggesting vasogenic edema. Vascular changes included patches of endothelial proliferation, and areas of telangiectasia. With 8000 rads, there had been impairment in VER from the second month and in appetite and motor function from the fifth month. The character and distribution of lesions were similar to those with 6000 rads, though less in number they had a greater variation in size and extended into the brain stem. At twelve months, with 4000 rads there were no changes. With 6000 rads there had been papilledema from the fourth to the sixth month, altered VER response accompanied by background delta activity from the sixth month and at sacrifice there were a few scattered areas of necrosis, with mineralization, and innumberable minute areas of calcium and iron deposits, along with widespread telangiectasia. With 8000 rads there had been similar clinical signs with the addition of optic atrophy and behavioral blindness. At sacrifice there were gross areas of coalescing necrosis with obvious brain swelling. In the remaining monkeys, with sacrifice dates 18 and 24 months from start of irradiation, the functional and structural observations will be completed in the coming year.

A definitive series will be started in July 1975 that will more closely simulate the human therapeutic experience and provide more precise information regarding the pathogenesis of the lesions. For this: 18 adult monkeys, all male, 10-12 Kg in weight have been selected to more exactly approximate in age the adult humans that are irradiated for malignant gliomas. A single fractionated dose of 6000 rads, that being therapeutically employed in at least ten medical centers in the United States, will be used over a course of six weeks. The exploratory effort, reported above demonstrated the lack of effect from 4000 rads and the highly destructive effort from 8000 rads. Alternate sides of the brain will be exposed on alternate days, with the output from the 20 MeV Linear accelerator at AFRRI brought closer to the energy level of the Clinical Linear Accelerators. The monkeys will be sacrificed in six sets of three, sequentially, from 8 weeks to 52 weeks following the start of irradiation, to gain a better concept of the pathogenesis of the delayed effects and the presence or absence of reparative processes.

Significance to Bio-Medical Research and the Program of the Institute: The principal advantage in simulating a therapeutic radiation regime in a monkey model is to observe the effects in a brain uncomplicated by pre-existing pathology, e.g. that resulting from a neoplasm, surgical trauma or chemotherapy. This kind of information will be useful in the planning of therapeutic efforts in man, with attention to "risk-benefit" factors, and in the interpretation of fresh neurological findings following therapy. There is a direct relevance to a major program of the Cancer Institute, i.e. the Brain Tumor Study Group, and we are privileged to share in their findings as this group is made aware of our results.

Key Descriptors: Irradiation - Monkey - Delayed effects - Brain damage-Vasogenic edema - Brain swelling - Regional cerebral blood flow - Radiation necrosis - Radiation therapy - Linear accelerator

3) Vietnam Registry

A registry for Head and Spinal Cord Injuries, as they occurred in military combat in Vietnam, was developed at the request of the Surgeon General of the U.S. Navy and was implemented with the cooperation of the Surgeons General of the U.S. Army and U.S. Air Force. The purpose was to insure uniformity of data collection and to identify cases for present and future studies. The yield from 1967 to 1970 was 2,043 entries that included 1,683 head injuries, 329 spinal cord injuries and 31 combinations of the two. The average age at time of injury was 21.6 years. A rigorous appraisal has demonstrated the uniformity and completeness of 1540 head injury forms. Ninety per cent of these were the result of explosively propelled missiles, 10% the result of vehicular accidents, falls, or blunt objects. In 85% there was local brain destruction, as evidenced by penetration of the dura with cortical laceration. 60% of these were accompanied by immediate loss in consciousness, by history. 30% were in coma, responding only to pain at time of examination, that was generally within six hours of injury. This provides an opportunity to group selected cases by regions of focal damage, with and without alteration in consciousness. These categories provide a background against which the natural history of sequelae may be determined.

A contract project proposal entitled, "Structural and Functional Sequelae of Penetrating Head Injury, Phase I", has been submitted to NINCDS, May, 1975. The conduct of this plan would update the clinical data on 1500 selected cases from the Registry, by a review of the accumulated Military and Veteran Administration hospital records since the time of Injury. The assembly of the records, abstracting the pertinent data, coding, transfer to magnetic tape, and analyses of sequelae, so identified, would be carried out in cooperation with the Follow-up Agency of the National Research Council, the Physical Sciences Laboratory of the Division of Computer Research and Technology, and three specially qualified professionals at the Eastern Virginia Medical School, Vanderbilt University Medical School and Harvard Medical School, respectively. Concomitant, with this would be the planning of Phase II, the in-hospital examination of three quarters of these patients with techniques not previously available, principal amongst which would be computerized axial tomography (EMI Scan) for the determination of brain loss. A second proposal would be required for Phase II.

Significance to Bio-Medical Research and the Program of the Institute: The observations during the acute phase of injury in these cases are more uniform and probably more accurate than any previous series of comparable size. This provides an extraordinary opportunity for studies of prognostic factors and the natural history of disabilities in central nervous system trauma in man.

Keyword Descriptors: Vietnam - Head Injury - Registry - Sequelae

Project No. Z01 NS 01693-07-LEN
1. Laboratory of Experimental Neurology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Functional and Structural Alterations Following X-irradiation of the Cerebral Cortex in the Monkey

Previous Project Number: Same

Principal Investigator: William F. Caveness, M. D.

Other Investigators: Hiroyuki Nakagaki, M. D.
Thomas L. Kemper, M. D.

Cooperating Units: Harvard University Medical School

Man Years

Total:	2.3
Professional:	1.1
Other:	1.2

Project Description

Objective: To determine the pathogenesis of early and late effects of irradiation on the central nervous system.

Methods Employed: In the model established several years ago, the right visual cortex, in the young adult *Macaca mulatta*, is exposed to 3,500 rads of x-irradiation in a single dose. Functional changes are monitored by evoked electroencephalographic responses from photic stimulation of the retina prior to and at biweekly intervals, following irradiation. The stimuli are programmed to provide single light flashes that may be averaged and a series of frequencies that range from three to thirty flashes per second in ten second epochs. The evoked response from the irradiated and non-irradiated visual cortex is led through a two-channel Grey-Walter, low frequency analyzer that provides histograms for the abundance of activity, or amplitude, for ten seconds at each of the frequencies employed. Thus one can compare directly the amplitude of the evoked response from the irradiated cortex with that from the homologous non-irradiated cortex. In addition, the frequency analyzer detects delta activity, one to three cycles per second, when it appears as a reaction to injury. The averaged evoked response of a series of thirty-two flashes at one second intervals has provided a sensitive indicator of alteration in wave form that is especially useful when the differences between the irradiated and non-irradiated side are less pronounced.

Sequential anatomical examinations have been carried out by sacrificing three animals at 72 hours, 1, 4, 12, 20, 28, 44 and 144 weeks for routine, Golgi-Cox and electron microscopic preparations. Six animals have served as controls.

Major Findings: In the first ten months the functional changes included an initial and secondary unilateral depression in the photic evoked response in the right visual cortex. There was ultrastructure evidence for changes in carbohydrate and protein metabolism within three days of exposure, alteration in the dendritic plexus within 4 to 12 weeks, and radionecrosis, in the exposed area, within 4 to 5 months following irradiation. (Special Issue of Brain Res. 7: 1-120, 1968) In nine additional preparations, long-term functional alterations, up to 36 months, have included bilateral depression of evoked response and behavioral blindness. The long-term structural changes have included periodic brain swelling, and retrograde transsynaptic degeneration within the visual system.

New Methods and Findings: During the preceding and current fiscal years, three new experimental procedures have been exploited to define the mechanisms operating in the long-term phenomena.

(1) The first consisted of twelve monkeys with implanted cerebral spinal fluid catheter-reservoir systems, permitting sequential monitoring of the CSF pressure following orthovoltage irradiation, with a single dose of 3,500 rads to the right occipital lobe. In eight animals there was relatively abrupt brain swelling, 18 to 35 weeks after irradiation, reflected by an increase in CSF pressure, 3-4 times baseline values, with absence of visual evoked response and behavioral blindness in four, and marked VER depression in four. Three had papilledema. Seven of these, sacrificed between 20 and 36 weeks, one hour after i.v. Evans Blue, 2 cc/kilo, showed proliferative and degenerative changes in the right occiput, deeply stained with dye. The swelling extended, beyond the irradiated and stained area, throughout the right hemisphere with distortion in brain structures. One sacrificed at 52 weeks, after resolution of the increased pressure and return of VER, had a fibrotic, non stained occipital lesion and no brain swelling. The remaining four animals showed variations in CSF pressure, seldom exceeding twice baseline, over 52 weeks; moderate reduction in the visual evoked response; and moderately swollen brains, with Evans Blue staining of the irradiation site, at sacrifice.

The significant feature in the delayed brain swelling was the breakdown of the blood brain barrier that accompanied the delayed radiation lesion, with the loss of serum fluid into the brain parenchyma. The impaired function was related to local destruction in the right visual cortex and widespread ipsilateral vasogenic edema and contralateral compression of the right and left visual pathways, respectively.

(2) A second series of 8 monkeys, prepared with a subarachnoid catheter-reservoir system and irradiated as described, was utilized to determine the changes in regional cerebral blood flow, by the ^{14}C antipyrine method, during the evolution and resolution of the vasogenic edema. There was found a reduction of blood flow in white and grey matter, first regionally, then throughout the ipsilateral hemisphere and finally, throughout the brain. This was accompanied by an increase in CSF pressure, CSF lactic dehydrogenase and total protein, and clinical signs of increased intracranial pressure. With resolution of CSF pressure, there was a return to baseline of CSF chemistry and partial resolution of the other parameters. The cerebral blood flow showed a greater recovery in grey than white matter, but there remained a diffuse depression suggesting a long-term impairment in cellular metabolism and/or blood flow regulatory mechanisms.

(3) Finally, the sequence of structural changes, proceeding from intracellular to local tissue, to remote and generalized effects, with remissions and exacerbations, strongly suggest isoimmune reactions and/or the activation of an infectious agent (slow and latent viruses). To test whether such a factor is present and transmissible, two newborn monkeys and four 100 day old monkey fetuses have received cerebral injections of material from the lesions in monkeys with postirradiation necrosis. Three monkeys have been immunosuppressed prior to and during irradiation to determine whether this will accelerate or decelerate the appearance and extent of the lesions. Electronmicroscopic evaluation of the irradiated brain and cervical lymph nodes in nine monkeys have not revealed recognizable viral particles but have shown consistantly tubuloreticular structures in endothelial cells, that have not been found in three controls. We believe these represent a cellular reaction to a virus or to an immune antigen.

Significance: The results from these investigations should have meaning and practical prognostic significance in the increasing human exposure to radiation in therapeutic efforts, the use of nuclear energy, and future space exploration.

Proposed Course: The studies following single exposure of the right visual cortex to 3,500 rads of orthovoltage irradiation with attention to the resultant brain swelling have been completed. (See publications). The continuation of the search for the basic mechanisms in the delayed breakdown in neural tissue following exposure to ionizing irradiation will include the completion of the histopathology of the monkeys subjected to immunosuppression and the monkeys innoculated with radiation lesions during fetal life. Note: This aspect of the radiation studies will be appended to the new project number Z01 NS 02159-01-LEN.

Keyword Descriptors: Irradiation - Monkey - Delayed effects - Brain damage - Vasogenic edema - Brain swelling - Regional cerebral blood flow - Radiation necrosis - Radiobiology.

Honors and Awards: None

Publications:

Caveness, W. F., Tanaka, A., Hess, K.H., Kemper, T.L., Tso, M.O.M., and Zimmerman, L.E.: Delayed brain swelling and functional derangement after x-irradiation of the right visual cortex in the macaca mulatta. Radiat. Res. 57, 104-120, 1974.

Tanaka, A., Ueno, H., Yamashita, Y., and Caveness, W. F.: Regional cerebral blood flow in delayed brain swelling following x-irradiation of the right occipital lobe in the monkey. Brain Res., In press, 1975.

Caveness, W.F., Kemper, T.L., and Vernon, M.L.: Is an infectious agent involved in the delayed effects of C.N.S. irradiation? Proceedings of the VII th Congress of Neuropathology, Budapest, 1974, In press.

Project No. Z01 NS 01694-07-LEN
1. Laboratory of Experimental Neurology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Ontogeny of Focal Seizures

Previous Project Number: Same

Principal Investigator: William F. Caveness, M.D.

Other Investigators: Yoriaka Yamashita, M.D.
Thomas L. Kemper, M.D.

Cooperating Units: Harvard University Medical School

Man Years:

Total:	2.0
Professional:	.8
Other:	1.2

Project Description

Objectives: To better understand the evolving seizure pattern in the developing brain.

Methods Employed: Focal seizures are induced by injecting 25,000 units of penicillin in 0.025 cc of aqueous solution into the hand-face area of the motor cortex of the Macoca mulatta at successive age levels from birth to young adulthood.

The onset and elaboration of the attack pattern is simultaneously recorded by electrocorticography, electromyography, and clinical observation.

The cerebra of the experimental, and a control, series of animals are examined for the level of development in cells, neuropil and myelin with attention to the motor cortex and subcortical tracts that are deemed relevant.

Major Findings: A significant feature of this investigation, in the Macaca mulatta, has been the apparent dependency of the immature motor cortex on subcortical structures in the development and propagation of the paroxysmal discharge.

To test this concept, the cortical-subcortical pathways beneath the face area in the precentral gyrus were sectioned in eight newborn monkeys. The undercut cortex was then injected with 25,000 units of penicillin. The paroxysmal activity recorded from an array of cortical electrodes developed within the undercut area and, except for limited and transient expression, did not spread beyond this region, suggesting a marked dependency upon the subcortical circuitry for propagation of the focal abnormality. Another procedure, in eight newborn animals, involved a supial section of the cortex on four sides that separated the face area from the remainder of the precentral gyrus and the frontal cortex anterior to the arcuate sulcus. When penicillin was injected into the circumsected cortex the paroxysmal activity developed and spread beyond the cortical sections in the superior and inferior motor strip and to other regions of the cortex with concomitant clinical expression of the seizure, suggesting a limited dependence upon the continuity of the cortex for propagation of the abnormal activity.

Each procedure was carried out in four monkeys at 24 months of age. The results, in contrast to the findings in the newborn, indicate a far greater dependence on the cortex for the development and propagation of the paroxysmal activity. After undercutting the site of activation, the propagation was unimpeded, but with circumsection of the activation site, the propagation was blocked, i.e. limited to the injection site, in the 24 month old monkeys.

During the past two years, the anatomical substrate for the seizure activity in the cortex has been extensively investigated with particular attention to the developing fiber systems, as delineated by Nauta techniques, and the developing pyramidal and stellate cells as demonstrated in Golgi preparations.

At birth, cortico-cortical connections are poorly developed, and cortico-subcortical circuits provide the major pathway for the propagation of cortical activity. In contrast, cortico-cortical connections are prominent at 24 months of age. This increase in cortical connectivity is reflected in the postnatal architectonic development of layers II and III, the loci of the most dramatic events of the postnatal development.

Significance to Bio-Medical Research and the Program of the Institute: The results from this investigation should improve the understanding and management of the age dependent seizure patterns in humans.

Proposed Course: These investigations have been completed and the results published in the scientific literature.

Keyword Descriptors: Monkey - Ontogeny - Focal fits

Honors and Awards: None

Publications: None

Project No. Z01 NS 01897-05-LEN
1. Laboratory of Experimental Neurology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Regional Blood Flow Studies

Previous Project Number: Same

Principal Investigator: William F. Caveness, M.D.

Other Investigators: Hideo Ueno, M.D.
Yoriaka Yamashita, M.D.
Louis Sokoloff, M.D.

Cooperating Units: Laboratory of Cerebral Metabolism, NIMH

Man Years:

Total:	2.0
Professional:	.8
Other:	1.2

Project Description

With the painstaking cooperation of the Laboratory of Cerebral Metabolism, we have developed a modification of the [^{14}C] antipyrine method for regional cerebral blood flow that permits the sectioning of the whole head for the autoradiograms and the magnification by five of the radiograms for dosimetry. This prevents distortion in the brain sections, and the densitometric measurement of small subcortical nuclear masses respectively.

To investigate the alteration in cerebral blood flow that accompanies the evolution and resolution of vasogenic edema that follows exposure to the cerebral cortex to ionizing irradiation, a series of monkeys was prepared with a spinal subarachnoid catheter-reservoir system prior to the exposure of the right occipital lobe to 3,500 rads of orthovoltage radiation. Utilizing the [^{14}C] antipyrine technique, the regional cerebral blood flow was determined at five discrete points on the CSF pressure curve; 1) baseline, 2) ascending, 3) peak, 4) descending, 5) complete resolution. A correlation of regional blood flow with the structural changes in the brain at each period was established.

A logical extension of the investigation of the propagation of focal seizures in the newborn monkey is a search for the subcortical circuits and nuclear masses that are involved in the seizure activity. In an attempt to determine the natural course of events, we have used the [^{14}C]antipyrine method at sequential intervals after the induction of a seizure.

Significance to Bio-Medical Research and the Program of the Institute: The investigation of regional blood flow alteration in brain edema following irradiation should add to our understanding of the effects of brain swelling, from whatever cause.

The global appraisal of the propagation of focal seizure activity by the regional cerebral blood flow method should provide a clearer understanding of the role of subcortical structures.

Proposed Course: The development of the rCBF methodology has been completed. Its application to date has been reported in project #1693 and its current and future application in project #2158.

Keyword Descriptors: [^{14}C]Antipyrine - Regional Cerebral Blood Flow - Focal Seizures - Brain Swelling

Honors and Awards: None

Publications:

Kato, M., Ueno, H., and Black, P.: Regional cerebral blood flow of the main visual pathways during photic stimulation of the retina in intact and split-brain monkeys. Exp. Neurol. 42, 65-77, 1974.

Project No. Z01 NS 01952-04-LEN
1. Laboratory of Experimental Neurology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Vietnam Registry, Analysis of Yield

Previous Project Number: Same

Principal Investigator: William F. Caveness, M.D.

Other investigators: Berkley L. Rish, CAPT (MC) USNR

Cooperating Units: Office of the Surgeon General, USN
Office of the Surgeon General, USA

Man Years:

Total:	1.0
Professional:	.5
Other:	.5

Project Description

Objectives: To insure uniformity of data collection in combat head injuries and to identify cases for present and future studies.

Methods Employed: A Registry for Head and Spinal Cord Injuries, as they occurred in military combat in Vietnam, was developed at the request of the Surgeon General of the Navy and was implemented with the cooperation of the Surgeons General of the Army and Air Force. The yield from field surgeons, 1967-1970, was 2,043 entries.

Major Findings: The Registry includes 1,683 head injuries, 329 spinal cord injuries and 31 combinations of the two. The average age at time of injury was 22.2 years. Of the head injuries, 90% were the result of explosively propelled missiles, 10% the result of vehicular accidents, falls, or blunt objects. In 85% there was local brain destruction, as evidenced by penetration of the dura with cortical laceration. 60% of these were accompanied by immediate loss in consciousness, by history. 30% were in coma, responding only to pain at time of examination, that was generally within six hours of injury. This provides an opportunity to group selected cases by regions of focal damage, with and without evidence of alteration in consciousness.

Significance to Bio-Medical Research and the Program of the Institute: The observations during the acute phase of injury in these cases are more uniform and probably more accurate than any previous series of comparable size. This provides an extraordinary opportunity for studies of prognostic factors and the natural history of disabilities in central nervous system trauma.

Proposed Course: A project proposal entitled, "Structural and Functional Sequelae of Penetrating Head Injury, Phase I", has been sumitted to NINCDS, May, 1975. The conduct of this plan would update the clinical data on 1500 selected cases from the Registry, by a review of the accumulated Military and Veteran Administration hospital records since the time of Injury. The assembly of the records, abstracting the pertinent data, coding, transfer to magnetic tape, and analyses of sequelae, so identified, would be carried out in cooperation with the Follow-up Agency of the National Research Council, the Physical Sciences Laboratory of the Division of Computer Research and Technology, and three specially qualified professionals at the Eastern Virginia Medical School, Vanderbilt University Medical School and Harvard Medical School, respectively. Concomitant, with this would be the planning of Phase II, the in-hospital examination of three quarters of these patients with techniques not previously available, principal amongst which would be computerized axial tomography (EMI Scan) for the determination of brain loss. A second proposal would be required for Phase II.

Keyword Descriptors: Vietnam - Head Injury - Registry

Honors and Awards: None

Publications:

Caveness, W.F., and Rish, B.L.: Structural and functional derangement in head injuries from Vietnam. An exhibit presented at the following meetings: American Association of Neurological Surgeons, April 1972, American Neurological Association, June, 1972, Congress of Neurological Surgeons, October, 1972, Association of Military Surgeons of the United States, December, 1972, and American Medical Association, June, 1974.

Project No. Z01 NS 02158-01-LEN
1. Laboratory of Experimental Neurology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Subcortical Factors in Experimental Focal Seizures

Previous Project Number: None

Principal Investigator: William F. Caveness, M.D.

Other Investigators: Hideo Ueno, M.D.
Yoriaka Yamashita, M.D.
Kenji Kosaka, M.D.
David M. Jacobowitz, Ph.D.

Cooperating Units: Laboratory of Clinical Sciences, Section on Histopharmacology
Harvard University Medical School

Man Years:

Total:	3.0
Professional:	1.8
Other:	1.2

Project Description

Objectives: To better understand the subcortical neuronal aggregates that are essential to the propagation of paroxysmal activity originating in the sensori-motor cortex.

Methods Employed: In the established animal model, focal seizures are induced by injecting 25,000 units of penicillin in 0.025 cc of aqueous solution 3 mm into the face-hand area of the right motor cortex of the Macaca mulatta at successive age levels from birth to young adulthood. The onset and elaboration of the attack pattern is simultaneously monitored by electrocorticography, electromyography and clinical observations.

A logical extension of these observations is a search for the subcortical circuits and nuclear masses that are involved in the propagation of the seizure activity. Previous studies by others have attempted to define "preferential" pathways by depth recording or by selective destruction of subcortical structures. For us it seemed wise to gain a global appraisal prior to the definitive study of individual elements. For this we have employed the [^{14}C] antipyrine technique for the determination of regional cerebral blood flow during selected phases in the propagation. The assumption, based on sound observations by others, is that an increase in neuronal activity will produce an increase in blood flow. The method may be summarized as follows: Approximately 200 μc/Kg of body weight of [^{14}C] antipyrine, a diffusable radioactive tracer, is continuously infused for 1 min via the femoral vein, during the development of a focal seizure. About 20 drops of arterial blood are collected from the femoral artery for the determination of the time course of the blood concentration of the isotope. One minute after the start of the infusion, the monkey is decapitated and the brain immediately immersed in a Freon bath chilled to -100 C in liquid nitrogen for 30 min, and temporarily stored at -20 C. In short, the fit is frozen in the brain. Subsequently, the brain is serially sectioned in the horizontal or the coronal plane at 30 μm thickness with a Jung K-type microtome at a cryostatically controlled temperature of -15 to -20 C. Approximately 100 sections from each brain are collected on plastic tape and dried on a hot plate at 60 C in preparation for macroautoradiographs. The slices are then placed on single coated blue sensitive medical X-ray film along with standard disc plates. The exposure time is 3-5 days and the films are developed by hand. The concentration of [^{14}C] antipyrine of selected anatomical components of the brain are calculated from the optical density of macroautoradiographs and of standard disc plates with an optical densitometric system that provides a fivefold magnification of cerebral structures.

From the concentration of the radioactive tracer in the arterial blood and the concentration in each anatomical component of interest, the value of the regional cerebral blood flow is determined by the formula of Kety.

Major Findings: In the series of yound adult monkeys, 8-10 Kg, during a phase of unilateral clinical seizure activity the following structures demonstrated a consistant increase in blood flow, 25+% greater than shown for the contralateral homologous structures: Right sensor-motor cortex (areas 4, 3-1-2), putamen, lateral and medial globus pallidus, substantia nigra, VL, VPL, VPM, CM of thalamus, pontine nuclei, and left cerebellar anterior and paramedian lobes, dentate and interpositus nuclei. Of particular interest was the marked increase, in a roughly somatotopic distribution, in the right putamen and globus pallidus and the left cerebellar cortex. A comparable pattern of increased subcortical activity was found in pubescent monkeys, 2.5-3 Kg.

Two advantages in exploring subcortical mechanisms in the newborn are being exploited. First, the level of development and integration of the cortex is such that the better developed subcortical systems are likely to have clearer expression. Secondly, the propagation of seizure activity, at this age, is protracted in time providing a better opportunity to observe the sequential involvement of neuronal aggregates. In the current series, the seizure activity is being interrupted at two and five minutes following the injection of penicillin. In the first the electroencephalographic paroxysmal activity has been sporadic and limited to the right sensori-motor area and the increased blood flow has been limited to the right sensori-motor cortex and the right putamen. In some of those decapitated five minutes after the penicillin injection, the abnormal electroencephalogram activity has also been limited to the right sensori-motor area but the increased blood flow has been found in the right sensori-motor cortex, supplementary motor cortex, putamen, globus pallidus and substantia nigra. Others, decapitated at five minutes have shown well organized 2-3/min EEG spikes from the right sensori-motor area along with concomitant movements in the contralateral hand. Increased blood flow was found in the right sensori-motor cortex, supplementary cortex, putamen, globus pallidus, substantia nigra, subthalamic nucleus, VL, VPL, VPM and CM of the thalamus, and the left anterior lobe of the cerebellum. Note: This newborn series shows a clear cut progression in subcortical structures with the progression in electroencephalographic activity along with the onset of clonic motor expression.

A second method, extending the primary objective is the determination of cathecholamines in conjunction with the blood flow studies. In the routine sectioning of the frozen head, when a structure of interest is exposed, first the section for the autoradiogram is taken, then a sample of the structure is obtained with a 18 gauge needle from the block remaining in the cryostat. The material is immediately transferred to the appropriate solutions for micro-assay of dopamine and norepinephrine or choline acetyl transferase. A small numerical ticket marks the defect in the block which is then photographed. These procedures are carried out faster than they can be described with minimal elevation in cryostat temperature.

Findings to date are limited to three, 3 Kg monkeys, and only a provisional comment is justified: The two seizure monkeys show an appreciable difference when compared to the single control, and in the two seizure monkeys there are appreciable unilateral changes. Further evaluations of both the method and the findings are being carried out.

A third method that is being employed is based on the prominence of the increased blood flow in the cerebellum during the seizure activity. The initial phase of this study involves the simultaneous recording of the electrical activity from the right and left cerebral cortex, hand-face areas, and the right and left anterior lobes of the cerebellar cortex.

The findings in ten 3 Kg monkeys clearly demonstrates a concomitant paroxysmal activity in the cerebral and contralateral cerebellar cortex.

Proposed Course: Continuation of blood flow studies with especial attention to the sequential involvement of subcortical structures in the newborn.

Continuation of the search for the role of neurotransmitters in the propagation of seizure activity, with consideration of indirect (enzymes, metabolites) as well as direct analyses.

Continuation of electrical studies of cerebellar-cerebral circuits, including the dentate, interpositus and VL of thalamus nuclei, that may play a key part in seizure propagation. Once established, the paroxysmal activity within these circuits will be interrupted with a cryoprobe.

Significance to Bio-Medical Research and the Program of the Institute: The results from these investigations should improve the understanding of subcortical factors in focal seizures and hopefully provide new approaches to therapy.

Keyword Descriptors: Focal Epilepsy - Subcortical - Nuclear masses- Paroxysmal propagation - Regional cerebral blood flow - Neurotransmitters - Depth recording

Honors and Awards: None

Publications: None

Project No. Z01 NS 02159-01-LEN
1. Laboratory of Experimental Neurology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Whole brain irradiation within the therapeutic range.

Previous Number: None

Principal Investigator: William F. Caveness, M.D.

Other Investigators: Hiroyuki Nakagaki, M.D.
Raymond R. O'Neill
W.E. Kiker, Ph.D.
Thomas L. Kemper, M.D.

Cooperating Units: Armed Forces Radiobiology Research Institute
Harvard University Medical School

Following the extensive studies of the delayed effects following a single exposure of 3,500 rads of orthvoltage radiation to a limited portion of the brain, a question that confronted us was: What, if any, of these effects would be observed if the exposure were divided and delivered to the whole brain in a manner similar to that utilized in standardized therapeutic procedures? Of particular interest was the protocol employed by the Brain Tumor Study Group, sponsored by the Cancer Institute. For their irradiation of malignant gliomas, they use a 6000 rads mid plane tumor dose over a period of 6-7 weeks with lateral parallel opposing fields. With megavoltage equipment, source axis distance of not less than 75 cm and a mid plane depth dose rate of not less than 33 rads per minute, daily increments of approximately 200 rads per day are delivered during a five day week. An exploratory effort in a monkey model is now being carried out.

Man Years

Total:	2.3
Professional:	1.1
Other:	1.2

Project Description

Objectives: To determine the effects on normal brain from super voltage radiation within the therapeutic range.

Methods Employed: Twelve Macaca mulatta, 3.0 Kg in weight, received 200 rads of super voltage radiation to the whole brain, each day, five days a week.

To bracket the human exposure of 6000 rads, now being used in at least 10 medical centers in the United States, three groups of four monkeys each received doses of 4000, 6000, and 8000 rads, respectively. The courses were four, six and eight weeks. The source was a linear accelerator with an electron beam energy of 20 MeV, that after collimation was converted to photons by tantalum foils and flattened by a lead disc to provide a nearly constant radiation field. Shielding with three to eight inches of lead provided protection except for an opening limited to the external borders of the brain, as determined by routine skull x-ray. Dosimetry by thermoluminescent dosimeters and ionizing chambers indicated a variation of less than 5% throughout phantom brains, irradiated unilaterally, from the left. The depth dose was comparable to that determined for bilateral exposure to 60 Cobalt. The depth rate at mid plane was 200 rads per minute The source-axis distance was 150 cm. The geometry of the exposure was determined at the Armed Forces Radiobiology Research Institute and the irradiations were carried out in that facility. Four additional monkeys served as controls.

One from each group has been sacrificed at 6, and 12 months; the remainder will be sacrificed at 18 and 24 months, from start of irradiation. The brains are fixed by perfusion with paraformaldehyde, embedded whole in celloidin, and cut in serial sections at a thickness of 35 micra. Every twentieth section is stained for myelin by the Loyez method and the adjacent section for nerve cells and glial nuclei with the Nissl method. An additional set, 100 sections apart, is stained with H & E and PAS, for vascular and other structures. Prior to and at each four weeks following the start of radiation, observations are conducted that include neurologic, funduscopic and electroencephalographic examinations.

Major Findings, to date: At six months, with 4000 rads there were no changes. With 6000 rads there had been depression in visual evoked response from the third month and at sacrifice 143 punctate lesions, one mm or less, were found, primarily in the corona radiata, centrum semiovale and internal capsule, being more frequent in the left hemisphere. These lesions were of different ages, the most recent having a necrotic core with a rim of macrophages, those older containing macrophages throughout, and the oldest showing mineral deposits. Myelin and adjacent nissl sections showed areas of palor exceeding the necrosis suggesting vasogenic edema. Vascular changes included patches of endothelial proliferation, and areas of telangiectasia. With 8000 rads, there had been impairment in VER from the second month and in appetite and motor function from the fifth month. The character and distribution of lesions were similar to those with 6000 rads, though less in number they had a greater variation in size and extended into the brain stem. At twelve months, with 4000 rads there were no changes. With 6000 rads there had been papilledema from the fourth to the sixth month, altered VER response accompanied by background delta activity from the sixth month and at sacrifice there were a few scattered areas of necrosis, with mineralization, and innumerable minute areas of calcium and iron deposits, along with widespread telangiectasia. With 8000 rads there had been similar clinical signs with the addition of optic atrophy and behavioral blindness. At sacrifice there were gross areas of coalescing necrosis with obvious brain swelling.

Significance to Bio-Medical Research and the Program of the Institute: The principal advantage in simulating a therapeutic radiation regime in a monkey model is to observe the effects in a brain uncomplicated by pre-existing pathology, e.g. that resulting from a neoplasm, surgical trauma or chemotherapy. This kind of information will be useful in the planning of therapeutic efforts in man, with attention to "risk-benefit" factors, and in the interpretation of fresh neurological findings following therapy. There is a direct relevance to a major program of the Cancer Institute, i.e. the Brain Tumor Study Group, and we are privileged to share in their findings as this group is made aware of our results.

Proposed Course: 1) Continuation of the monitoring of functional and structural changes that follow whole brain super voltage irradiation in those with fractionated doses of 4000, 6000, and 8000 rads, respectively, with sacrifice at 18 and 24 months.

2) The initiation of a new series in July 1975 that will more closely simulate the human therapeutic experience and provide more precise information regarding the pathogenesis of the lesions. For this: 18 adult monkeys, all male, 10-12 Kg in weight have been selected to more exactly approximate in age the adult humans that are irradiated for malignant gliomas. A single fractionated dose of 6000 rads, that used in human therapy, will be used over a course of six weeks. The exploratory effort, reported above demonstrated the lack of effect from 4000 rads and the highly destructive effort from 8000 rads. Alternate sides of the brain will be exposed on alternate days, with the output from the 20 MeV Linear accelerator at AFRRI brought closer to the energy level of the Clinical Linear Accelerators. The monkeys will be sacrificed in six sets of three, sequentially, from 8 weeks to 52 weeks following the start of irradiation, to gain a better concept of the development of the delayed effects and the presence or absence of reparative processes.

Keyword Descriptors: Radiation therapy - Brain Damage - Delayed effects - Linear Accelerator - Monkey

Honors and Awards: None

Publications:

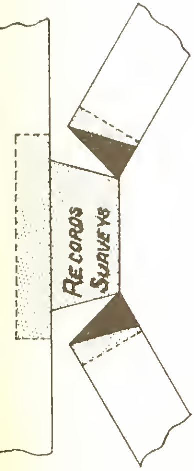
Nakagaki, H., Brunhart, G., Kemper, T. L., and Caveness, W. F.: Monkey Brain Damage from Radiation in the Therapeutic Range, J. Neurosurg. In press, 1975

HOW TO USE
THESE SEPARATORS

Use one page for each separation.

Select appropriate tab, add further identification if desired, and cover it with scotch tape.

Cut off and discard all tabs except the one covered by tape.



TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

ANNUAL REPORT
July 1, 1974 through June 30, 1975
Laboratory of Neurochemistry, Intramural Research
National Institute of Neurological and Communicative
Disorders and Stroke

R. Wayne Albers, Acting Chief

The projects within the LNC are directed to quite diverse problems in neurobiology. The unifying concern is that of explaining neural mechanisms in molecular terms. Thus each of the several independent scientists benefits from the ready availability of a wide range of techniques and expertise which results from this diversity.

Each of the four Sections reports substantial results from the efforts of the past year.

The Section on Enzyme Chemistry reports completed studies which indicate that Mg^{++} has a direct role in the ionophoric part of the Na^+ and K^+ active transport mechanism. New studies have begun to define the factors which are rate-limiting in the ATPase reaction which energises the active transport of Na^+ and K^+. In addition a newly discovered phosphorylated component of the ATPase-containing membranes is being investigated to determine whether this component is directly related to the transport system. In a different area of research, this section has developed a convenient model for producing astrocytic gliosis in rat spinal cords which should provide material for future biochemical studies.

The Section on Physiology and Metabolism reports on the existence of enzymes of the plasma membrane which act on substrates in the external medium. The work centers on enzymes which degrade nucleotides. Earlier evidence has indicated that cells secrete ATP which in turn can decrease membrane permeability to a variety of substances. These studies have been extended to include the further metabolism of adenosine. Another project in this Section involves the study of neurotransmitter metabolism in marine animals. This has included participation in an expedition to British Columbia which studied the phenomenon of rapid deterioration in Pacific salmon after spawning. This has been viewed as an accelerated aging process. In addition to the neurotransmitter studies, a peculiar dependence of salmon heart muscle on stored triglycerides as an energy reservoir has been elucidated.

The Section on Neural Development and Regeneration reports projects in two principal areas: (i) the trophic effects of peripheral nerves on target organs and (ii) the immunological factors which determine the viability of neuronal transplants. The latter project, aside from its intrinsic importance, is a practical problem, the solution of which is necessary to permit further studies on nerve regeneration and neurotrophic factors.

Preliminary results are reported from a study which is designed to determine whether the fusion of myoblasts into muscle fibers may involve a selection of phenotypic variants which could determine the pathway of differentiation into "red" and "white" muscle fibers and thus limit their potential for neurotrophic regulation. At present the evidence favors random fusion of phenotypically variant myoblasts and thus emphasizes the role of the neurotrophic factors.

The immunological studies have determined that treatment of neonatal rats of one strain with bone marrow from another strain can permit neural transplants between these strains, a procedure which is otherwise unsuccessful. Efforts to develop a technique for maintaining cultures of relatively differentiated CNS tissue would provide an important tool to evaluate the role of neurotrophic factors in the CNS.

The Section on Amino Acids and Electrolyte Metabolism reports on two principal areas. One complements studies in the Section on NDR and involves isolation and characterization of a protein from nerve extracts which possesses trophic activity on muscle cells grown in culture. Active trophic material has been obtained from spinal cord, brain and sciatic nerve tissues. The material is a protein of high molecular weight. It appears to accelerate the growth of myoblasts and their rate of fusion and differentiation.

The second major project in this Section is directed toward the processes which regulate cation balances in brain. These studies complement the studies on active sodium transport in the Section on EC. The phenomenon of hibernation provides one of the most extreme examples of physiological adjustment which involves cation balance as a major factor. These studies have established that an important factor in adapting to hibernating conditions is the ability of these animals to regulate the lipid composition of their cell membranes. This in turn influences the kinetic properties of the Na^+ transport system and permits it to function more efficiently at low temperatures.

All of the above projects have resulted in major publications and reflect the continuing vitality of the Laboratory. One of the senior staff will leave at the end of this year to accept a major university appointment. This loss, together with that which resulted from Dr. Tower's appointment as Institute Director, requires serious consideration of the possibilities for enlarging the staff of senior scientists. A separate report on this issue has been submitted to the Scientific Director.

Project No. Z01 NS 02076-02 LNC
1. Neurochemistry
2. Amino Acids & Electrolytes
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on Trophic Substances of Nerve.

Previous Serial Number: Same.

Principal Investigator: T. H. Oh, Ph.D.

Other Investigators: None.

Cooperating Units: None.

Man Years:

Total:	1.8
Professional:	1.0
Other:	0.8

Project Description:

Objectives: The overall objective of this project is to determine the chemical nature of trophic factor and its mechanism of action. Experiments are planned to isolate trophic factor from nerve extracts, which is responsible for the differentiation, maturation, and long-term maintenance of muscle cells grown in culture. The use of tissue culture to study the trophic interactions between nerve and muscle is a relatively new and unique investigational model system.

Background. The accumulated data indicating that spinal motor nerves have an influence on morphological development, membrane acetylcholine sensitivity, distribution and activity of cholinesterase, speed of contraction, and biochemical profile of skeletal muscle was reviewed by Guth (1968; 1969) and more recently by Drachman (1974). Also, recent reports have shown that many *in vivo* observations of trophic influences of nerves can be reproduced in nerve and muscle cells grown in culture (Kano and Shimada, 1971; Lentz, 1971; Peterson and Crain, 1972; Oh *et al*., 1972).

Three separate reports indicate that substances containing neurotrophic properties can be extracted from nerve tissues. Lebowitz and Singer (1970) observed that nerve homogenates infused into denervated limbs of adult newts stimulated protein synthesis. Lentz (1971) found that when forelimb muscle explants from adult newts were maintained in the culture media containing nerve homogenates, cholinesterase activity of muscle was greater than in untreated muscle cultures. Oh et al. (1972) demonstrated that acetylcholinesterase activity of muscle cells was significantly increased by addition of brain-spinal cord extracts to the culture media. The active substances in these extracts were found to be protein molecules (Singer, 1974; Lentz, 1974; Oh, 1975).

Recent evidence has shown that colchicine, known to block axoplasmic flow, produces denervation-like changes in muscle without impairment of normal neural transmission and muscle activity (Albuquerque et al., 1972; Hoffman and Thesleff, 1972; Cangiano, 1973). These results thus indicate that neurotrophic effects are independent of nerve transmission, and further suggest that the trophic factor is transported by axoplasmic flow.

Methods: During the past several years the methodology for culturing nerve and muscle has been adapted for studying the trophic interactions between nerve and muscle, and considerable information concerning the interactions has been obtained in my laboratory.

The procedures for tissue cultures of nerve and muscle cells have been previously described in detail (Kim et al., 1972; Oh and Johnson, 1972; Oh et al., 1972; Oh, 1975). Methodology developed for the isolation of a neurotrophic protein is described under Major Findings.

Major Findings: When dissociated skeletal muscle cells were cultured, acetylcholinesterase activity of muscle cells was significantly increased by the presence of spinal cord explants that innervated the muscle fibers, by spinal cord explants that did not innervate the muscle, or by addition of brain and spinal cord extracts to the culture media (Oh et al., 1972). These in vitro observations are consistent with the view that innervation influences the development of acetylcholinesterase in muscle. The significant increase in acetylcholinesterase in muscle cells exposed to non-innervated spinal cord explants indicates that a physical contact between nerve and muscle is not essential for the trophic regulation of acetylcholinesterase in muscle, and in addition, that the trophic effect is mediated by a diffusible chemical substance produced by nerves. The increase in acetylcholinesterase in muscle cells grown in the presence of brain and spinal cord extracts also suggests that the substance containing neurotrophic properties can be extracted from nerve tissues.

The chemical nature of the active substance in the brain and spinal cord extracts (chick embryos) was further analyzed. When muscle cells were maintained in the presence of the extracts, the morphological development, protein synthesis and acetylcholinesterase activity of muscle were stimulated (Oh, 1975). Boiling or heating the extracts to 60°C abolished their activities. The protein nature of the active substance in the extracts was further suggested by the results of enzyme degradation of the extracts. The activity was significantly reduced by trypsin and protease treatment, but was resistant to neuraminidase. The molecular size of the active substance was estimated by filtering the extracts through Diaflo membranes. A filter cake of Diaflo membrane (type XM-300) was found to be most effective on muscle cells suggesting that the active substance in the extracts is a protein with a molecular weight greater than 300,000 daltons.

Also, the mature muscle fibers required the presence of nerve extracts to be maintained for long periods of time (up to 8 weeks) in culture.

Preliminary experiments showed that adult sciatic nerve and brain extracts stimulated the DNA and protein synthesis, acetylcholinesterase activity and morphological development of muscle cells grown in culture. Also, the sciatic nerve extract was more effective than the brain extract at the same concentration, suggesting that the neurotrophic factors are axoplasmic flow materials.

During hibernation, skeletal muscle of animals undergo some denervation-like changes, such as loss of weight, spread of acetylcholine sensitivity, and decrease in m.e.p.p. frequency at neuromuscular junctions (Lyman and O'Brien, 1969; Moravec et al., 1973). Furthermore, Moravec et al. (1973) suggested that interruption of axoplasmic flow of the neurotrophic factor to the nerve endings may lead to the induction of denervation-like changes during hibernation. Preliminary experiments were carried out to see whether this hypothesis is valid. Nerve extracts were prepared from normal and hibernating hamster sciatic nerve and spinal cord tissues, and added to the culture media of muscle cells. Addition of normal hamster sciatic nerve and spinal cord extracts stimulated the DNA and protein synthesis of muscle, while the extracts of hibernating hamster sciatic nerve and spinal cord did not produce such effects. The results thus suggest that a lack or limitation of the neurotrophic factor may be the cause of denervation-like changes in muscle of hibernating hamsters. If this is true, the hibernating animals could be a new investigational model for studying denervation or disuse effects on skeletal muscles.

Significance: The long-term objective is to accumulate data on the biochemical profile of nerve-muscle interactions to facilitate future studies on certain neuromuscular diseases, such as muscular dystrophy, myasthenia gravis and muscle atrophy in which normal interactions are impaired.

It is well established that motor nerves influence the growth and differentiation of skeletal muscle fibers. This is undoubtedly an important factor in normal development and perhaps also in adaptive responses of muscles to use. Recent evidence suggests that a lack or limitation of a trophic factor produced by nerve may be the cause of some forms of muscular dystrophy.

Trophic effects of nerves probably also act upon other nerves within the central nervous system. Thus the nerve-muscle system serves as a relatively accessible model for comparable CNS interactions.

Proposed Course: The principal investigator will leave the laboratory July 1, 1975 and the project will be terminated in the Laboratory of Neurochemistry to be continued at the University of Maryland.

Key Words: neurotrophic factor.

Honors and Awards: None.

Publications:

1. Oh, T. H.: Neurotrophic Effects: Characterization of the Nerve Extract that Stimulates Muscle Development in Culture. Exp. Neurol. 46: 432-438, 1975.

Project No. Z01 NS 02077-02 LNC
1. Neurochemistry
2. Amino Acids & Electrolytes
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Physiological and Biochemical Correlates of Cation Balance in Brain.

Previous Serial Number: Same.

Principal Investigator: S. S. Goldman, Ph.D.

Other Investigators: None.

Cooperating Units: None.

Man Years:

Total:	1.0
Professional:	1.0
Other:	0

Project Description:

Objectives: To elucidate the biochemical basis of the regulation of cation balance in brain.

Methods: Cation balance in brain is considered to be the resultant of two categories of events: (1) anabolic processes, primarily active transport, which create a non-equilibrium distribution of electrolytes; (2) catabolic processes, such as membrane excitation and passive leaks, which permit electrolytes to redistribute toward equilibrium. These two categories can generally be distinguished on the basis of their responses to temperature: anabolic processes have high temperature coefficients while catabolic processes, as here defined, have a low temperature dependence.

The strategy employed in this research is to compare the electrolyte metabolism and related processes in warm adapted hamsters with those of cold adapted (hibernating) hamsters. To this end a stock of hibernating hamsters is maintained.

Specific Methods: A working hypothesis of the current project is that alterations in membrane lipids are central to the cold-adaptation phenomenon. To this end biochemical analyses of membrane lipids are employed. These include thin layer and gas chromatographic methods.

Standard methods of enzyme isolation and assay are used for the hamster ($Na^+ + K^+$)-ATPase characterization. The TLC separation of nucleotides and subsequent assay of radioactivity is employed in the method for the nucleotide exchange reaction. ^{32}P-ATP is employed to measure enzyme phosphorylation.

Fluorescence spectroscopy and fluorescence polarization are employed for studies of brain membrane physical properties which may change in the hibernating state.

Major Findings: In the past year it has been found that a lipid adaptation occurs during hibernation. This adaptation occurs in a direction that would favor a relatively more fluid membrane at low temperature. Biochemically a decrease in cholesterol and an increase in the content of mono-unsaturated fatty acid was reported. This lipid adaptation helps to explain the acclimation that was observed for brain Na^+-K^+-ATPase. A detailed kinetic analysis of this ATPase was conducted and recently completed. It was concluded that the temperature sensitive sites as well as the acclimation sites of this enzyme are those which involve the conformational changes that occur within the ATPase catalytic cycle. The efficacy by which these conformational changes occur are dependent upon the physical characteristics of the lipid matrix of the membrane.

Significance: Electrolyte balance is a central aspect of normal brain physiology. In addition, cerebral edema and epilepsy are major pathological problems related to electrolyte imbalance. The use of hibernation as a tool in this study also relates to the clinical use of induced hypothermia for surgical procedures. At present the use of this technique is limited primarily by the inability to control neurological damage related to cerebral edema. The hibernating animal has evidently solved this problem and incorporated the solution into its normal physiology.

Thus, the maintenance of a fluid membrane at low temperature is of prime importance in maintaining the integrity of the animal during hibernation. This brings us back to the original objectives and significance of the project. The ability to maintain electrolyte balance in normal brain is as stated previously, dependent upon both active and passive forces acting in concert with one another. Employing the hibernatcr as a tool to investigate these forces may give us a unique opportunity to better appreciate the limits that are imposed upon the central nervous system in man during states akin to induced hypothermia, cerebral edema and other states that affect cation balance.

Proposed Course: A physical-chemical analysis of the properties of the cell membrane will be conducted to establish how the lipid and enzymatic (Na^+-K^+-ATPase) acclimation that occurs during hibernation enables the animal to resist the effects of cold. Factors unique to hibernators, the hamster in particular, will be investigated. These include investigations on fatty acid desaturase, an enzyme of which little is known about its role in adult brain and nothing is known about the enzyme in a hibernator.

An estimation of the microviscoscity of brain cell membranes will be made employing fluorometric techniques. It has been reported that highly apolar fluorochromic probes can be incorporated into both synthetic and natural membranes. Comparing its fluorescence characteristics to known synthetic systems, an estimation of the microviscoscity of the membrane can be made in both the warm adapted and hibernating hamster.

Key Words: hibernation, cation balance in brain, Na^+-K^+-ATPase, membrane fluidity, membrane viscoscity, hypothermia, membrane lipids.

Honors and Awards: None.

Publications:

1. Goldman, S. S.: Cold Resistance of the Brain during Hibernation. III. Evidence of a Lipid Adaptation. Am. J. Physiol. 228: 834-838, 1975.

Project No. Z01 NS 00813-14 LNC
1. Neurochemistry
2. Enzyme Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Enzymological Aspects of Neural Function.

Previous Serial Number: Same.

Principal Investigator: R. W. Albers, Ph.D.

Other Investigators: D. Jean, Ph.D.; A. C. Swann, M.D.; A. Hobbs, Ph.D.; G. J. Koval; J. Froelich, Ph.D.

Cooperating Units: Section on Molecular Chemistry, Laboratory of Molecular Aging, NIA.

Man Years:

Total:	5.4
Professional:	2.8
Other:	2.6

Project Description:

Objectives: To assess the functions, molecular structures and mechanisms of enzymes and enzyme systems which characterize neural tissues or which respond to neural influences. Current studies are directed toward elucidation of the mechanism and structure of the (Na^+-K^+)-ATPase which is the enzymatic expression of active Na^+ transport and hence of the membrane potential generating system of neural tissues.

The experimental objectives with respect to the (Na^+-K^+)-ATPase are:

(1) to obtain the enzyme in purified form and to elucidate the structure and catalytic properties of the purified enzyme;

(2) to determine the influences of other components of the cell membrane upon this enzyme;

(3) to determine the factors in the normal cellular environment which regulate active Na^+ and K^+ transport;

(4) to assess possible involvement of this system in certain pathologies.

Methods: The general methodology for isolation and purification of the ATPase has been reported previously and published. Extensive work in both steady-state and transient-state reaction kinetics has been performed over the past year. This involves spectrophotometric and radioisotopic measurements. The DCRT MLAB computer program has been invaluable in analysing these data through a laboratory-based remote graphics terminal.

A preparative-scale gel electrophoresis system we designed specifically for application to the detergent-solubilized membrane proteins which are of interest in this study (Jean & Albers). This continues to be a vital part of our procedure for obtaining pure enzyme and enzyme subunits for use as antigens in our immunochemical studies.

Major Findings relating to the $(Na^+ + K^+)$-ATPase:

(a) The role of Mg^{++} in the $(Na^+ + K^+)$-ATPase reaction.

Steady-state kinetics studies have been directed toward the role of Mg^{++} in this reaction. Evidence for 3 different Mg^{++} binding sites were obtained. Analysis of the results indicate that Mg^{++} must associate and dissociate once in each catalytic cycle. This is compatible with a role for Mg^{++} in the conformational cycle and probably also in the ionophoric events.

(b) The interaction of lithium and rubidium ions with the ATPase.

Steady-state kinetic studies have provided additional and very direct evidence for the existence of two distinct sites (high and low affinity) for K^+ activation of the reaction cycle. These may be equated with outward and inward facing K^+ ionophores.

(c) Transient state kinetics of $(Na^+ + K^+)$-ATPase.

These studies are in collaboration with Dr. J. Froehlich of the Section on Molecular Aging, NIA-NIH. Dr. Froehlich is equipped to perform "rapid-quenching" experiments which permit us to measure the rate of phosphorylation or dephosphorylation of the enzyme. The time resolution of the apparatus is of the order of 4 milliseconds. Utilizing the electric eel enzyme and ^{32}P-ATP, we have measured simultaneously the rates of appearance of inorganic ^{32}P. The first studies have examined the transient kinetics at rate-limiting concentrations of ATP. We have obtained evidence that under these conditions the rate of formation of phosphoryl enzyme is not limiting the overall hydrolytic reaction. The dissociation of an enzyme-bound form of inorganic phosphate appears to be the slowest step. Moreover, the concentration of ATP regulates the rate of dissociation of this bound phosphate.

(d) "Slow" phosphorylation of an electroplax membrane protein.

Electric organ membranes can be phosphorylated by ATP at a site which is distinct from the $(Na^+ + K^+)$-ATPase intermediate. This is a relatively slow reaction which requires Mg^{++} but is not markedly influenced by Na^+ or K^+. The phosphorylated protein has characteristics which indicate that a serine or threonine residue is the probable acceptor. The protein which is phosphorylated has a molecular weight very close to that of the glyco-peptide subunit of the ATPase. At this time the two proteins have not been resolved.

(e) Immunological studies of the subunit structure of the $(Na^+ + K^+)$-ATPase.

Antisera to the soluble Electrophorus enzyme and to its major constituent polypeptides have been produced in rabbits. These antisera have been tested against ATPase preparations from several other species with negative results. A quantitative fluorescence assay has been developed to determine the titer of these sera. This work is preparatory to a collaborative study on the fine structure of electroplax membranes which will be outlined below.

Significance: The $(Na^+ + K^+)$-ATPase is the biochemical expression of active Na^+ and K^+ transport in most animal cells. As such, it is the molecular basis for the development of electrical potentials across cell membranes, for the regulation of cell volume, water and electrolyte balance and a spectrum of coupled transport systems involving vital metabolites, neurotransmitters, etc.

This area of research has attracted a large number of workers (more than 80 citations of work from this Section appeared in 1974). Much of the current effort is directed to questions of molecular mechanism: the nature and number of the ionophores in the enzyme; the nature of the protein conformational changes in the reaction cycle; the modes of regulation of transport activity. At the same time a variety of possible roles of this system in the etiology of different pathologies are being explored: epilepsy, myotonias, denervation atrophy, slow virus encephalopathy, etc.

The applied studies are particularly dependent upon the basic studies in this area, since the abnormal states of this system are only detectable in relation to the known parameters of the "normal" system. Any genetic defect, for example, which would inactivate the Na^+ transport system to an extent that would be obvious in a simple enzyme activity assay would undoubtedly be lethal. However, it is already apparent that certain subtle changes in the enzyme or its membrane environment may be compatible with "normal" enzyme activity under standard conditions, and yet induce abnormal function under different conditions (See report under S. Goldman).

Proposed Course:

(a and b) At the present time the steady-state kinetic studies are either in press or in manuscript and new studies in this area are not contemplated in the coming year.

(c) The transient-state studies will be extended to determine the effects of the important ligands and inhibitors on rates of phosphorylation and dephosphorylation of the ATPase (Froehlich and Albers).

(d) The studies of the subunit structure of the enzyme will continue. The immediate effort in this area will be to determine the relationship between the "stable" phosphopeptide and the glycoprotein subunit of the ATPase (Koval and Albers).

(e) An investigation of the structure and orientation of the ($Na^+ + K^+$)-ATPase within the cell membrane will be initiated using an approach which will employ electron microscopic examination of the electroplaque membrane and the peroxidase technique for antibody localization. The current speculation is that the glycoprotein subunit may be associated with the outer surface of the membrane whereas the larger subunit may be either at the inner half or extend entirely across the plasma membrane (Jean, Albers and other collaborators).

Key Words: active transport, ($Na^+ + K^+$)-ATPase, sodium, potassium, electric organ.

Honors and Awards: None.

Publications:

1. Jean, D. H., Albers, R. W., and Koval, G. J.: Sodium-Potassium-Activated Adenosine Triphosphatase of Electrophorus Electric Organ X. Immunochemical Properties of the Lubrol-solubilized Enzyme and its Constituent Polypeptides. J. Biol. Chem. 250: 1035-1040, 1974.
2. Kennedy, W. R., Webster, H. De F., Yoon, K. S., and Jean, D. H.: Human Muscle Spindles: Microfilaments in the Group A Sensory Nerve Endings. Anatomical Record 180: 521-532, 1974.
3. Shamoo, A. E., Myers, M. M., Blumenthal, R., and Albers, R. W.: Ionophoric Material Derived from Eel Membrane Preparations. I. Chemical Characteristics. J. Membrane Biol. 19: 129-140, 1974.
4. Swann, A. C., Daniels, A., Albers, R. W., and Koval, G. J.: Interactions of Lectins with ($Na^+ + K^+$)-Adenosine Triphosphatase of Eel Electric Organ. Biochim. Biophys. Acta, in press.
5. Swann, A. C., and Albers, R. W.: Sodium-Potassium-Activated ATPase of Mammalian Brain: Regulation of Phosphatase Activity. Biochim. Biophys. Acta 382: 437-456, 1975.

Project No. Z01 NS 02075-02 LNC
1. Neurochemistry
2. Enzyme Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biochemical Regulation in Astrocytes.

Previous Serial Number: Same.

Principal Investigator: R. W. Albers, Ph.D.

Other Investigators: D. H. Jean, Ph.D.

Cooperating Units: None.

Man Years:

Total:	0.4
Professional:	0.2
Other:	0.2

Project Description:

Objectives: To elucidate the common biochemical denominator of the astrocytic response to chemical and physical insults to the brain. The principal focus of this project is the mechanism which "turns on" and sustains this response.

Methods: In addition to the approaches outlined in the previous report, our preliminary work has permitted us to adopt some specific methods. The method of choice for producing astrocytosis at this time appears to be the use of 6-aminonicotinamide in rats. A method of direct injection of this agent into the spinal cord (subdurally) permits the use of drug doses which produce hind-leg paralysis after 2 days and extensive astrocytic gliosis in the ventral gray matter within two weeks. The animals are otherwise healthy and can be maintained for several months when necessary.

Major Findings: The major finding is the method for producing gliosis described above. Preliminary biochemical work has been performed on control and experimental tissue but the efforts are still exploratory.

Significance: The astrocyte response occurs in demyelinating diseases and physical or chemical injury. If the injury is chronic a dense scar of fibrous astrocytes may form. This is sometimes associated with epileptic foci and the formation of these scars may often be a factor in the inability of central neurons to regenerate their normal connections after brain or spinal cord injury.

Proposed Course: We intend to compare the levels of a number of enzymes and proteins in normal and gliosed spinal cord tissue. Ornithine decarboxylase and the levels of associated polyamines appear to be likely candidates since this system is known to show increased activity during cellular proliferation. Astrocytes manufacture a specific protein which is thought to be related to the fibrous structures within the reactive cells. Protein patterns will be examined.

A number of cellular control mechanisms are related to the protein phosphokinase system. Therefore, we plan to examine the pattern of ^{32}P incorporation into proteins in normal and gliosed tissue. The sampling problem is still a serious obstacle in this research. It may be necessary to resort to microdissection to obtain sufficiently pure samples of astrocytes. Experimental findings of interest will be followed up by an examination of human pathological specimens such as glial scars and M.S. plaques.

Key Words: astrocytes, gliosis, 6-amino-nicotinamide.

Honors and Awards: None.

Publications: None.

Project No. Z01 NS 01054-12 LNC
1. Neurochemistry
2. Neural Development & Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Trophic Functions of the Peripheral Nervous System.

Previous Serial Number: Same.

Principal Investigator: Lloyd Guth, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.8
Professional:	0.5
Other:	0.3

Project Description:

Objectives: To determine whether the formation of slow and fast muscle fibers results from the selective fusion of "slow" and "fast" clones of myoblasts.

Methods: Muscles were obtained from two inbred strains of mice, one of which was homozygous for the AA form of glucose phosphate isomerase and the other was homozygous for the BB form of this enzyme. The slow and fast muscles from both strains were excised and minced in order to induce degeneration of the adult muscle fibers and to stimulate the division and fusion of the satellite cells (myoblasts). Various combinations of slow and fast minces were placed on the chorioallantoic membrane of the chick embryo and allowed to differentiate. After 18 days of culture the specimens were removed and analyzed for isozyme content by starch gel electrophoresis.

Major Findings: The results to date are preliminary, but in most cases AA, AB, and BB isozymes were found in each preparation.

Significance: This indicates that each syncytial muscle fiber contained nuclei that derived from both strains of mice. Consequently, there is no evidence that myoblast fusion is selective and no evidence for a clonal derivation of slow and fast muscle myoblasts.

Proposed Course: Dr. Guth is retiring from the NIH and the project is to be discontinued.

Publications:

Guth, L.: Trophic Functions. Pages 329-343 (Chapter 11) in "The Peripheral Nervous System," edited by John I. Hubbard. New York: Plenum Publishing Corp., 1974.

Project No. Z01 NS 01303-10 LNC
1. Neurochemistry
2. Neural Development & Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Elucidation of factors regulating the metabolic and functional characteristics of skeletal muscle sensori - motor components.

Previous Project No.: Same and incorporating Serial No. NDS(I)-66 LNC/NDR 1303

Principal Investigator: Herbert Yellin, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.1
Professional:	1.0
Other:	0.1

Project Description:

Objectives:

1. Attempt to transplant and sustain a) segments of spinal cord; b) spinal ganglia; and c) skeletal muscle, in the anterior chamber of the eye, and maximize conditions for their interaction.

2. Attempt to discern the responsivity of the muscle units of extraocular muscles to alien-innervation.

3. Attempt to distinguish the role of individual nuclei, of the multinucleate skeletal muscle fiber, in the growth and differentiation, as well as the metabolic and functional remodeling of muscle under circumstances of unusual use or experimental manipulation (e.g., cross-innervation).

4. Attempt to establish the nature of the functional interrelationships of the proprioceptive Golgi tendon organs with the several types of metabolically and functionally disparate motor units of mammalian skeletal muscle.

Methods:

1. Using inbred (isogenic) strains of rats, transplanted small segments of lumbosacral spinal cord of neonatal and adult rats to the anterior chamber of the eye. In some instances the spinal cord transplant was accompanied by a small skeletal muscle. Several months after surgery the transplants were studied for their general morphological features using hematoxylin and eosin, for Nissl substance with thionine, for cell bodies and processes with silver stains and reactions for cholinesterase, for myelin with PAS-Hem., and for mitrochondrial succinic dehydrogenase.

2. Difficulty encountered thus far in exposing as well as denervating and reinnervating specific extraocular muscles of the rat.

3. Utilized Acridine-Orange fluorescence staining to study DNA and RNA of multinucleate skeletal muscle fibers in frozen sections of normal rat musculature. Must devise appropriate method, compatible with the staining procedure, whereby individual, teased muscle fibers and their nuclei can be studied with fluorescent technique.

4. Alternating serial sections of skeletal muscle have been examined by stains for connective tissue (collagen) and muscle fiber type (ATPase).

Major Findings:

1. Segments of the spinal cord of neonatal rats can be successfully transplanted to the anterior chamber of the eye. Spinal ganglia of both neonatal and adult animals can likewise be sustained. Occasionally skeletal muscle has been successfully sustained in the anterior chamber of the eye, in the presence of spinal cord. The use of isogenic strains of animals precludes graft rejection for up to six months (the duration of the study).

2. Occulomotor nerve and its branches are relatively inaccessible and relatively unsuited for microsurgical manipulation in the rat.

3. The nuclei of mammalian extrafusal muscle fibers, as well as those of associated cells, fluoresce yellow or yellow-green in normal muscle, with the remainder of the tissue relatively unstained. Provision must be made for discerning betwixt muscle satellite cell nuclei, muscle nuclei, and fibrocyte nuclei.

4. Preliminary efforts have demonstrated that the muscle fibers inserting into Golgi tendon organs are of at least two varieties of muscle units. [Burke et al, '71 have described spectrum of muscle unit-types which are grouped for convenience into "fast-fatiguing," "fatigue-resistant," and "slow" muscle units.]

Significance:

1. The anterior chamber of the eye of inbred strains of animals is an excellent locale in which to "culture" mammalian spinal cord (and presumably other central nervous system constituents). The spinal cord graft must be at a relatively early stage in its development, in order to survive. Thereafter the cord segment will remain viable for at least six months, exhibiting typical properties. The staining properties of the myelin, as well as other constituents of the "cultured" cord indicate that a maturation of the once neonatal (near fetal) cord has occurred. The "in-oculo" preparation of neurons and neuropil is simple and self-sustaining, as opposed to the typical in-vitro tissue culture method. Excluding certain limitations imposed by the presence of an intact vascular supply (i.e., a blood supply containing cellular and humoral elements), the "in-oculo" preparation appears to lend itself to observing a variety of interactions between central, as well as peripheral neuronal components and their varied sensory and motor effector organs.

2. Unable, as yet, to demonstrate whether the "unusual" external musculature of the eye, which may contain tonic, graded contraction muscle fibers, as well as phasic, twitch fibers, is capable of manifesting metabolic conversion as a consequence of altered use or alien-innervation. (Altered use or alien-innervation does alter the metabolism of the general somatic musculature). The degree of mutability of the extraocular muscles may be of significance with respect to our understanding of specific disorders characterized by atypical positioning and/or movement of the eyes.

3. Determine whether all, or a percentage of myonuclei, of the multinucleate muscle fiber, are necessary for reprograming the metabolism of a muscle fiber subsequent to altered use or alien-innervation.

Many neuromuscular disorders, the myopathies in particular, are characterized not only by deterioration of the skeletal musculature, but by seemingly uncontrolled and inappropriate efforts at muscle fiber regeneration. The relative involvement of a muscle may depend upon the nature (phenotype) and number of specific myonuclei within individual muscle fibers.

4. Determine whether all of the principal types of muscle units (fast-fatigue, fatigue-resistant, and slow units) are attached directly in-series with the Golgi tendon organs. The tendon organ is generally considered to exert an inhibitory effect on all homonymous motoneurons (i.e., the neurons controlling the muscle in which the tendon organs are present). However, a preferential in-series alignment of specific types of muscle units with tendon organs could exist. Further clarification of the relationship of motor units to tendon organs (as well as to other feedback systems, e.g., spindles) would improve our understanding of the sensori-motor mechanisms involved in 1) normal posture and movement, 2) shivering and physiological tremor, and 3) abnormal conditions characterized by tremor, rigidity, and other dystonias.

Proposed Course of Project: To continue these studies and:

1. Determine optimal conditions for implanting spinal cord in the anterior chamber of the eye. Further, to successfully interact spinal cord with spinal ganglia and skeletal muscle in the anterior chamber of the eye and thereby achieve a "self-sustaining culture" of inter-dependent tissues.

2. Attempt to demonstrate the enzyme-histochemical (metabolic) mutability or immutability of extraocular muscle units.

3. Learn to tease mammalian muscle fibers in conjunction with Acridine-Orange staining for DNA and RNA. Ultimately to determine the role of the full complement of myonuclei in sustaining and/or remodeling the metabolism and function of skeletal muscle.

4. Elucidate fully the metabolic and functional types of muscle units in-series with the Golgi tendon organs.

Key Words: Transplantation, central nervous system; Myonuclei; Tendon organs; cross-innervation.

Honors and Awards: None

Publications:

Yellin, H.: Spindle Induction and Differentiation in Murine Dystrophy. Experientia 30: 286-287, 1974.

Yellin, H.: Muscle Cross-Reinnervation. Nature 249: 596, 1974.

Yellin, H.: Limitations to the Neuroregulation of Enzymes in Mammalian Skeletal Muscle. Anat- Rec. (In Press--Fulfills objective 1 of '74 report; to discern the mutability of skeletal muscle units innervated by cranial, as opposed to spinal nerves.)

Riley, D.A.: Factors Affecting the Conversion of Cross-Reinnervated Skeletal Muscles. Amer. J. Anat. 140: 609-615, 1974. (Fulfills objective 2 of '74 report. Dr. Riley (UCSF) was formerly a Muscular Dystrophy Foundation investigator at the N.I.N.D.S. and performed preliminary studies leading to the above communication while in the Section of Neuronal Development and Regeneration.)

Project No. Z01 NS 01586-08 LNC
1. Neurochemistry
2. Neural Development and Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Regeneration and trophic function of neurons in the peripheral nervous system.

Previous Project No.: Same and incorporating Serial No. NDS(I)-68 LNC/NDR 1586

Principal Investigator: Andrew A. Zalewski, M.D.

Other Investigators: Willys K. Silvers, Ph.D.

Cooperating Units: Department of Human Genetics, University of Pennsylvania

Man Years

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description:

Background of Project: The purpose of this project is to investigate the mechanisms by which injured neurons regenerate their nerve fibers and to evaluate neuronal and extra-neuronal factors that may influence the establishment of functional connections. To this end, regeneration studies are being performed in order to investigate nerve fiber growth, trophic nerve function, and any selectivity or plasticity that may exist between the neuron and its appropriate end-organ. The interaction occurring between neurons and taste buds is being used as a model for the project since taste buds develop under the influence of the nerve, the nerve is required for the maintenance of the buds, and because the buds disappear after denervation and reappear after nerve fiber regeneration.

Pertinent Past Studies: Previous results demonstrated that some neurons in sensory ganglia could survive after transplantation to the anterior chamber of the eye and when tongue grafts were added with them nerve fibers from the ganglia grew into the tongue grafts and caused the regeneration of taste buds. By substituting different ganglia (sensory or sympathetic), different tissue grafts (tongue and skin) and by varying the age of the grafts (neonatal and adult, or combining neonatal with adult) various aspects of selectivity and plasticity of the neurons and taste buds were revealed.

Last year I reported on immunological studies of transplanted neurons which were performed by using homografts rather than isografts (i.e., grafts between animals of a highly inbred strain which due to their genetic similarity do not evoke an immune response) of sensory ganglia. These studies showed that neurons in homografts of ganglia could survive if the recipient animal was rendered immunologically tolerant (see Methods for details of producing tolerance) and that these neurons could function neurotrophically in that they regenerated nerve fibers into tongue grafts (isografts) and caused taste buds to form. Other studies showed that sympathetic and parasympathetic neurons and satellite and Schwann cells also survived in tolerant recipients. Unless recipients were made tolerant neurons and supporting cells in homografts were immunologically rejected. I proposed to continue with the immunological studies and performed the following experiments.

Objectives:

1. Determine the duration of immunological tolerance.

2. Determine the specificity of immunological tolerance.

3. Determine whether neonatal neurons could be successfully homografted.

4. Determine whether central nervous system (CNS) neurons (adult and neonatal) could survive transplantation.

Methods Employed: Rats of the Brown Norway (BN) and Lewis (LE) strains were used. These animals are highly imbred but different genetically so that grafts exchanged between them are immunologically rejected. Tolerance was induced in Brown Norway rats by injecting them at birth with (LE X BN)F_1 bone marrow cells. At birth the immune system of the BN rat is not fully developed so that the injected LE bone marrow cells are not recognized as foreign and hence not rejected. As a result of exposure to LE antigens subsequent transplantation of LE grafts to the neonatally-treated BN rats survive.

1. In order to study the duration of tolerance, a group of 70-75 day old neonatally-treated BN rats received LE sensory ganglia (nodose) placed into each eye and each sternomastoid muscle. Some LE grafts were examined 35-180 days after transplantation and compared to LE grafts given to normal (untreated) BN rats. After all LE grafts were removed the BN rats who bore them were allowed to age 12-14 more months and then they were again challenged with LE ganglia placed intramuscularly. This later study was performed to determine whether removal of the original LE grafts influenced the tolerant state of the animal with regard to time and subsequent LE ganglia grafts.

2. The specificity of tolerance was tested by making BN rats tolerant with (LE X BN)F_1 bone marrow cells and then grafting them individually with LE ganglia or with ganglia from genetically different rat strains. Isogenic Lewis (LE) and Buffalo (BUF) as well as hybrid (LE X BN)F_1 animals were used as graft donors. Grafts were examined 35 and 100 days after transplantation.

3. The ability of neonatal homografts of ganglia to survive was investigated by homografting newborn LE sensory and sympathetic ganglia to normal or tolerant BN rats. In addition BN tongue grafts were added to sensory ganglia to see if any surviving neurons could induce taste bud regeneration. The neonatally-treated BN rats were injected with adult rather than neonatal (LE X BN)F_1 bone marrow cells.

4. The ability of CNS neurons to survive was determined by transplanting grafts of various regions of adult or neonatal brain and spinal cord. CNS isografts were used since the first objective of this study was to determine which CNS neurons could survive transplantation and where their optimal transplantation site was.

All grafts were examined by light microscopy using frozen sections stained in suitable ways to determine the presence of neurons, nerve fibers, taste buds, and cellular immune responses.

Major Findings:

1. All neurons in homografts of ganglia in normal (untreated) BN rats were rejected within 35 days. In contrast many neurons survived in homografts in tolerant BN recipients. The number of neurons present was essentially the same at 35 and 180 days and compared favorably to the number present in isografts (from previous studies). Neurons were also present after 60 days in tolerant BN rats who were regrafted with LE ganglia (after removal of all original LE grafts) whereas untreated BN rats, who were now sensitized to LE grafts, rejected LE ganglia in 14 days.

2. BN rats rendered tolerant by (LE X BN)F_1 bone marrow treatment accepted LE ganglia at 35 and 100 days but rejected Buffalo and (LE X BUF)F_1 hybrid ganglia at 35 days.

3. Adult (LE X BN)F_1 bone marrow cells were able to induce tolerance of LE neonatal sensory ganglia in BN rats. Thus neonatal sensory neurons survived in tolerant BN rats but were rejected in normal BN rats. Furthermore, surviving LE neonatal neurons matured and were able to induce taste bud formation in tongue grafts. On the other hand, no neurons were found in neonatal sympathetic ganglia after transplantation to normal or tolerant BN rats. This result was not due to a differential induction of tolerance with regard to sensory and sympathetic neurons but rather it was due to the inability of sympathetic neurons to withstand the trauma of transplantation (i.e., nerve fiber transaction and grafting without vascular anastomosis). This was subsequently demonstrated by performing isograft studies with neonatal sympathetic ganglia. It was found that although neonatal sympathetic neurons could initially survive grafting they progressively degenerated and disappeared over a 3-week course. This loss of sympathetic neurons was not associated with any appreciable cellular inflammation.

4. Several attempts were made to isograft pieces of adult cerebral cortex, cerebellum, and spinal cord. Unfortunately, all neurons in these adult CNS grafts disappeared within a week whether placed into the eye or into muscle. Presently neonatal CNS grafts are being employed and in collaboration with Dr. Yellin from our section it was found that neonatal spinal cord can survive grafting. Other CNS grafts (e.g., cerebellum) will be employed too as experiments are being performed to determine how CNS neurons are effected by an immune response and if tolerance to them can be induced.

Significance:

1. Immunological tolerance seems to be a method of immunosuppression which insures the permanent survival of neurons in homografts of ganglia. Furthermore, regrafting is possible. (Data to be published)

2. Immunological tolerance is specific and only those ganglia grafts survive which are fully compatible with the genotype of the tolerance inducing bone marrow cells. (Data to be published)

3. Immunological tolerance can be induced to neonatal homografts of sensory ganglia and surviving neurons can form functional connections (i.e., induce taste bud formation). The failure of neonatal sympathetic neurons to serve transplantation demonstrates the vulnerability of different type of neurons (sensory and sympathetic) to injury even though they are the same age and subjected to the same trauma. The observation that adult (LE X BN)F_1 bone marrow cells can induce tolerance to neonatal LE sensory neurons means that qualitatively the histocompatibility antigens expressed on adult cells are represented on neonatal sensory ganglia cells and that the neonatal cells possess no significant embryonic histocompatibility antigen that are detrimental to homograft survival. (Data to be published)

4. The failure of adult CNS isografts to survive whereas neonatal CNS (spinal cord--see Dr. Yellin: cerebellum--recent literature) grafts do again emphasizes the vulnerability of different aged neurons to injury. It is interesting to speculate that inadequate amounts of growth substances or failure to achieve a functional innervation may be factors which act in conjunction with injury effects (e.g., nerve fiber transaction, hypoxia, inflammation, etc.) to kill certain traumatized CNS or PNS developing (neonatal) neurons. In this regard it would be pertinent to determine whether neonatal sympathetic neurons could survive transplantation if (1) nerve growth factor were given, (2) the transplantation site (eye) was denervated (sympathectomy), or (3) both therapies were performed.

Proposed Course of Project:

A) Immunological studies:

1) Determine the immunological response to homografted CNS neurons and whether tolerance to CNS grafts can be induced if rejection occurs. One aim of this study is to use spinal cord grafts to promote spinal cord regeneration. A cord graft could act as a "bridge" which regenerating cord fibers might use to

reach the cut ends of the cord. Isografts of cord can be used as free-grafts in rats but it would be highly desirable to do this experiment in larger animals too where vascular anastomosis of the graft might be attempted. Since inbred strains of large animals are not available homografts of cord will eventually be used and hence the need to know the effects of an immune response on CNS tissue.

2) Attempt to induce immunological tolerance in adult animals. See (1) for reasons.

3) Compare conventional immunosuppressive methods (ALS serum steroids, antimetabolites (azothioprin) with tolerance induction in the event (2) fails and for reasons in (1).

4) Determine whether better genetic matching (typing) between donor and recipient allows tolerance to be induced easier and whether better functional results (nerve fiber regeneration and taste bud development) occur. In this study Ag-B compatible rats (Lewis and Fischer strains) will be used rather than the Ag-B-histoincompatible Lewis-Brown Norway combination used previously. For discussion of histocompatibility see Exp. Neurol. 33:576-583, 1971.

5) Determine whether reflex connections can be established with homografted ganglia in tolerant recipients.

6) Determine effects of autoimmune disease on trophic nerve function. An autoimmune peripheral neuritis will be induced by injecting nerve emulsified in Freund's adjuvant, ascertaining that the nerve to buds are involved and determining what effect the neuritis has on trophic nerve function (i.e., maintaining the buds).

7) Determine effects of autoimmune salivary glands disease on taste buds. It has been reported that patients with Sjogren's syndrome suffer from taste abnormalities and loss of taste buds. Furthermore, it is postulated that a zinc protein from salivary glands is needed to help the nerve maintain taste buds. My interest in this observation stems from my finding (Data to be published) that taste buds have appeared in the epithelium of the ducts of von Ebner's salivary gland after transplanting tongue grafts to the eye. This gland lies just below the tongue's vallate papilla which contains taste buds and pieces of it are unavoidably transplaced with the tongue grafts. Can a zinc-protein in the ducts be acting as a stimulus to attract regenerating nerve fibers?

8) Determine the immunological response to homografts of nerve. It would be useful to know how histocompatibility and tolerance influence the repair and subsequent function in the neurosurgical treatment of traumatic peripheral nerve injury, that is one where a large segment of nerve is destroyed.

B) Peripheral nerve regeneration:

1) Attempt to see if the spinal cord environment is conducive to nerve fiber regeneration over long distances. A peripheral nerve or nerve fibers from a transplanted ganglion will be implanted into the transected spinal cord in various regions in order to utilize degenerating ascending and descending pathways and determine over what if any distance these nerve fibers regenerate. With regard to spinal cord regeneration it would be pertinent to know if nerve fibers crossed the cut ends of the cord that they could grow the appropriate distances up and down the cord. This attempt would include sympathetic nerve fibers since catecholamine fibers have recently been shown to be capable of regenerating after lesions in the brain, an observation which has generated renewed interest in CNS regeneration. Catecholamine fluorescent histochemical studies would be useful here.

C) Neuron-taste bud interactions:

1) Attempt to grow taste buds in tissue culture. This has been attempted but results of others have been discouraging. We have begun such studies in conjunction with Dr. Oh (Section on Amino Acids and Electrolytes) who is skilled in tissue culture techniques. The problem here is finding conditions in which both ganglia and tongue tissue survives. We have cultured ganglia successfully for 4 weeks but are having difficulties with the tongue tissue. We hope to compare taste buds grown in culture and in the eye to see which model would be better suited for pharmacological studies.

2) Test specificities of epithelium and connective tissue in forming taste buds. By trypsinizing tongue and skin we hope to do recombination studies to see if specificities reside in connective tissue or epithelium. For example, will taste buds form in a recombinant of tongue connective tissue and skin epithelium reinnervated by the nodose ganglion?

3) Attempt to hyperinnervate tongue or skin grafts by using two or more ganglia to see if greater numbers of nerve fiber increase the number of buds in tongue grafts or now will induce them in skin grafts (one ganglion did not on previous studies).

4) Determine by what mechanism the trophic nerve influence is delivered to the tongue tissue. Presumably it is via axoplasmic flow and by selectively blocking fast or slow flow with colchicine some information about what or how the neurotrophic influence (presumably it is chemical) is transmitted might be gained.

Key Words: Neuronal transplantation, immunological tolerance, taste buds, nerve regeneration.

Honors and Awards: None.

Publications:

1. Zalewski, A. A.: Trophic Function of Neurons in Transplanted Neonatal Ganglia. Exp. Neurol. 45: 189-193, 1974.
2. Zalewski, A. A.: Neuronal and Tissue Specifications Involved in Taste Bud Formation. Ann. N.Y. Acad. Sci. 228: 344-354, 1974.

Project No. Z01 NS 02074-02 LNC
1. Neurochemistry
2. Neural Development & Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Regeneration in the Central Nervous System.

Previous Serial No.: Same

Principal Investigator: Lloyd Guth, M.D.

Other Investigators: R. Wayne Albers, Ph.D.; Amiram Daniel, Ph.D.

Cooperating Units: Surgical Neurology Branch, IR-NINCDS

Man Years

Total:	1.1
Professional:	0.5
Other:	0.6

Project Description:

Objectives: To develop improved methods for the histological analysis of injury and repair of the central nervous system.

Methods: A new method for preparing CNS tissues for enzyme histochemistry has been developed and a new staining technique for such important enzymes in the CNS as $(Na^+ + K^+)$-activated ATPase and choline acetyltransferase have been developed.

Major Findings: The light microscopical localization of these enzymes has been accomplished with greater resolution than before. The ATPase is localized to the neuropil of the gray matter of the spinal cord and the choline acetylase is found mainly at the presynaptic terminations on the ventral horn cells. Some subcellular fractions (synaptosomes and synaptosomal mitochondria and vesicles) do not.

Significance: This is a technical advance of considerable importance to the histochemistry of the nervous system, and it now makes it possible to study the CNS with the great variety of histochemical methods that have been developed and perfected over the past twenty years.

Proposed Course: The project is discontinued because the principal investigator is retiring from the NIH.

Publications:

Guth, L., and Albers, R. W.: Histochemical Demonstration of ($Na^+ + K^+$)-Activated Adenosine Triphosphatase. J. Histochem Cytochem. 22: 320-326, 1974.

Guth, L.: Axonal Regeneration and Functional Plasticity in the Central Nervous System. Exp. Neurol. 45: 606-654, 1974.

Daniel, A., and Guth, L.: Histochemical Demonstration of ($Na^+ + K^+$)-Activated ATPase Activity of Synaptosomes and Synaptosomal Membranes. Exp. Neurol. 47: 181-188, 1975.

Project No. Z01 NS 01480-08 LNC
1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Metabolism of Neurohumoral Transmitter Substances in Marine Animals.

Previous Serial Number: Same.

Principal Investigator: E. G. Trams, Ph.D.

Other Investigators: A. A. Benson, Ph.D.; S. Patton, Ph.D.; E. A. Brown, Ph.D.

Cooperating Units: Scripps Institute of Oceanography, La Jolla, California;
Div. of Food Science, Pennsylvania State University;
Pulmonary Branch, NHLI.

Man Years:

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: To exploit for the study of neurobiology the great variety and abundance and the biochemical specialization of oceanic life. In particular we have selected to study some aspects of neurochemistry in marine organisms where certain tissues, species or phenomena constitute an evolutionary amplification of normal human physiology.

Methods: Our studies have been concerned with the metabolism of plasma membranes and transmitter substances. The methodology in use is adjusted to specific requirements according to current laboratory techniques. Use is made of radioactive precursors, i.e. labeled biogenic amines are introduced for studies of their metabolism in vivo or in vitro. Common enzymological approaches are used when appropriate, especially in drug metabolism, toxicology, and when the effects of putative transmitters on metabolic pathways are investigated.

Experimental animals or organisms used: various kinds of Selachians, Torpedinidae, several species of Teleosts, in particular Electrophorus electricus and Pacific salmon (Oncorrhyncus spp.); small crustaceans, Ecchinoderms, Aplysia. Emphasis has been placed on the study of membranes, electric organs and nervous tissues or tissues which are of interest to our principal program on structure and function of excitable membranes.

An unusual method for us was that we were able to conduct metabolic studies on explanted, beating salmon hearts. We used a perfusion system of our own design. These experiments were conducted during the R/V "Alpha Helix" Expedition. They probably constitute a first attempt for such experiments aboard a ship.

Major Findings: We had the privilege to participate in the 1974 R/V "Alpha Helix" Expedition to British Columbia. The aim of the expedition was to investigate metabolic patterns in the migrating and spawning Pacific salmon (Oncorrhyncus spp.). Our particular studies involved an assessment of brain ATPases and 5'-nucleotidase activity during the final life stages of the salmon. There is evidence that the Pacific salmon can serve as a model for the study of the aging process. We had previously reported that, in the CNS of the migrating fish, certain metabolic changes might be associated with the degenerative processes which occur in this species (E. G. Trams, Nature 222: 492-493, 1969). We collected brains from salmon caught in salt water at the very onset of the spawning migration, from fish during the migration and entry into fresh water streams, and from fish which were spawning or were spawned out and moribund.

There appeared to be no changes in the brain 5'-nucleotidase activity. Na^+, K^+ stimulated ATPase of salmon brain was generally only from 25 to 40 per cent of total brain ATPase activity. The overall pattern for the ATPases showed a decline as the salmon approached the spawning beds. Variations between individuals, however, were large and the data spread made a meaningful comparison difficult.

In collaboration with Dr. Brown (NHLI) we conducted assays of salmon interrenal body acetylcholinesterase. Activity of this enzyme appeared to decline while the gland underwent a marked initial hypertrophy and eventually (after spawning) a nearly complete involution. Individual variations again precluded any evaluation which delineated the apparent pattern more precisely.

In collaboration with S. Patton we surveyed organ lipid composition in a number of fish species with the aid of thin-layer chromatography. The relatively high levels of triglycerides (TG) in heart muscle of migrating salt-water salmon seemed impressive and we therefore made a more extensive study of the lipid composition in salmon hearts as compared to that in hearts of other local fishes. Triglycerides (TG) were substantially elevated in heart lipids of pre-spawning Pacific salmon as compared to other marine fish sampled in the waters of British Columbia, Canada. In vivo tracer studies with ^{14}C-palmitate showed that FFA was largely converted into TGFA in the heart of migrating salmon. In spawned-out salmon a stroking reduction of TG in heart lipids revealed another facet of metabolic collapse of this species during the reproductive cycle. It involved virtually complete loss, on average 94%, of heart triglycerides during the fresh water migration to the spawning grounds. In vivo palmitate-1-^{14}C incorporation into heart triglycerides, as well as heart slice incubations, revealed the triglyceride pathway as an active metabolic route even in post-spawning fish that were only hours from death. The heart took up and retained fatty acid from the serum more readily than liver, skeletal muscle or brain. Following intravenous injection of the label, maximum specific activity of heart triglycerides was reached in approximately one hr. The rapid turnover of the glyceride pool coupled with its diminishing mass is evidence of a substantial fatty acid flow through the TGFA in the heart muscle.

We suggested that FFA may not be directly available to the muscle from the plasma compartment. Plasma fatty acids appeared to be converted to TGFA in the heart muscle prior to utilization in the cardiac energy household. Whether or not this constitutes an obligatory pathway or only a metabolic shunt, mostly activated during energy crises, should be the subject of further experimentation.

Significance: These investigations emphasize the usefulness of the marine environment in the search for models of molecular biological mechanisms or diseases. In addition, comparative studies often lead to a better understanding of certain structures or functions of a biosystem.

Our collaborative study (with S. Patton and A. A. Benson) on the lipid metabolism of salmon heart muscle has revealed that the major energy source of the heart muscle, fatty acids, are available to this tissue only as triglyceride fatty acids. This is in contrast to the classical notion that the main energy source of muscle is free fatty acid. We have postulated that this unusual pathway in cardiac muscle may be obligatory and constitutes an energy cushion (reservoir) which affords the heart additional protection in times of high demands or overloads.

Proposed Course: We will continue our comparative studies in the field of neurobiology and the biochemistry of plasma membranes.

Key Words: marine biomedical research, CNS dysfunction in aging salmon, cardiac metabolism.

Honors and Awards: None.

Publications:

1. Patton, S., and Trams, E. G.: Salmon Heart Triglycerides during Spawning Migration. Comp. Biochem. Physiol. 46B: 851-855, 1973.
2. Patton, S., Zulak, I. M., and Trams, E. G.: Fatty Acid Metabolism via Triglyceride in the Salmon Heart. J. Mol. Cell. Cardiol., in press.

Project No. Z01 NS 01481-08 LNC
1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on the Composition and Metabolism of Cellular Membranes.

Previous Serial Number: Same.

Principal Investigator: E. G. Trams, Ph.D.

Other Investigators: W. Reichert, M.D.; Carl J. Lauter.

Cooperating Units: None.

Man Years:

Total:	2.7
Professional:	1.8
Other:	0.9

Project Description:

Objectives: To elucidate the inter-relationship between the molecular composition of the plasma membrane and the functions that macromolecular building blocks serve in transport, bioelectrogenesis and intercellular communication.

Methods: We continued the propagation of various tissue culture cell lines in the laboratory. Established clones of mouse neuroblastoma (N-18), human astrocytoma (Cox Clone), human oligodendroglioma and neonatal hamster (NN) astrocytes were carried in routine monolayer cultures. In addition, we have begun to develop cell cultures from neonatal mouse brain. These monolayer cultures have served for the study of ecto-enzymes of the plasma membrane. We also have used the cell cultures as sources for plasma membrane preparations and as enzyme sources for a variety of metabolic studies concerned with the role of adenine nucleotides as modulators of cell permeability. Changes of membrane permeability (and possibly excitability threshold) were investigated in monolayer cultures which had been pulse labeled with a variety of isotopic ions.

In an extension of these studies we have measured the activity of Mg^{++} and Na^{+}, K^{+}-dependent ATPases in brains of two strains of mice, one of which is peculiarly sensitive to the induction of audiogenic seizures (DBA/2N). The activity of the ATPases in the DBA/2N mice was compared with the activity in C57 mice (controls). Induction of seizures by sound exposures was accomplished by established procedures. Enzyme activity was measured on homogenates of whole brain and in subcellular fractions (microsomes and synaptosomes).

In pilot experiments we have also measured ATPase activities in brains of chimpanzees and mice which had established degenerative lesions of Kuru, Creutzfeldt-Jacob and Scrapie pathogenesis. Estimation of monoamine-oxidase activity in the affected areas served as a control.

Major Findings: In our publication "On the Sidedness of Plasma Membrane Enzymes" (Biochim. Biophys. Acta 345: 180-197, 1974) we documented our findings on the transverse asymmetry of certain plasma membrane marker enzymes. In particular we had emphasized that 5'-nucleotidase and Mg^{++} ATPase were Ecto-enzymes. On the basis of our observations we had predicted that these two enzymes would display characteristics of ecto-enzymes in most eukaryotic cells. A series of recent publications from several other laboratories tends to confirm our contention (J. W. DePierre and M. L. Karnovsky, Science 183: 1096-1098, 1974; A. C. Newby, J. P. Luzio and C. N. Hales, Biochem. J. 146: 625-633, 1975; P. Madel, V. Stefanovic, P. Mandel and A. Rosenberg, Transactions Am. Soc. Neurochem., 6th Ann. Meeting 1975, p. 102).

Additional studies conducted by us during this reporting period have shown that not only a 5'-nucleotidase and a Mg^{++} ATPase, but also an inorganic pyrophosphatase exhibited characteristics of an ecto-enzyme (Biochim. Biophys. Acta 370: 556, 1974). This observation was also confirmed recently (Stefanovic, Mandel and Rosenberg, 1975). Following this line of inquiry we also explored the possibility that adenosine deaminase functioned as an ecto-enzyme. Translocated cytoplasmic ATP was suspected as the primary substrate for ecto-ATPase and further catabolism via ecto-5'-nucleotidase had been demonstrated by us. Since conversion of adenosine (obtained by the action of ecto-5'-nucleotidase on 5'AMP) to inosine was very rapid in monolayer cultures, we have studies the metabolism of adenosine in detail. Our results have shown, however, that the bulk of extracellular adenosine was degraded by a soluble cytoplasmic adenosine deaminase after rapid transport into the cell interior. The products of the reaction (inosine, hypoxanthine) were equally rapidly transferred back into the medium, and the entire sequence thus simulated the action of an ecto-adenosine deaminase. We have actually demonstrated the presence of a plasma membrane adenosine deaminase, but its activity was insignificant by comparison with the soluble cytoplasmic enzyme. We are pursuing some aspects of this study, since they appear to be relevant to the extrusion of adenosine from electrically stimulated brain tissue (I. H. Heller and H. McIlwain, Brain Res. 53: 105-116, 1973; I. Pull and H. McIlwain, Biochem. J. 136: 893-901, 1973).

It has become clear that the ecto-enzyme constituted a very active catalytic system on the cell surface and we endeavoured to gain some insights into their function. We have extended last year's studies on the effects of ectopic ATP on plasma membrane permeability. The effect is temperature dependent and not specific with regard to the permeant ion or molecule. Only nucleoside triphosphates exhibited this activity and other phosphoesters were without effect. Different cell lines displayed significantly different dose responses to added ATP. Neonatal hamster astrocytes responded to as little as 5×10^{-7} M ATP but the N-18 neuroblastoma required 5×10^{-4} M ATP.

In vivo the ATP-induced rise in membrane permeability (or excitability threshold) is presumably terminated by ecto-ATPase. A change in ecto-ATPase activity or a congenital (or induced) deficiency of this enzyme would have a profound effect on the ionic environment of a cell population. Such a lesion might be particularly critical in the CNS. We have conducted some experiments based on the working hypothesis that the molecular basis of certain convulsive disorders was due to a change in ecto-ATPase activity.

The effect of sound exposure and audiogenic seizures on cerebral ATPase was investigated using two strains of mice, DBA/2N and C57Bl/6N. Using non-exposed littermates as controls, a protocol of sound treatments began when the animals were 16 or 21 days old, and most treated mice received two sound exposures separated by at least 48 h. Seizures were significantly more frequent in DBA/2N (86% overall) than C57Bl/6N (24% overall). Mg^{++}- and Na^{+}-K^{+}-ATPase activities were assayed in homogenate and microsome fractions from mice sacrificed at 24-25 or 30-31 days old and in synaptosomes from mice sacrificed at 25-28 days old. A minimum of 48 h separated the last sound exposure and sacrifice. Control versus sound comparisons were made using data standardized by litter. A statistically significant decrease of microsomal ATPase was found for the Mg^{++}-dependent enzyme activity of sound exposed 24-25 day old DBA/2N and for the Na^{+}-K^{+}-dependent enzyme activity of sound exposed 30-31 day old C57Bl/6N. Enzyme activities were not significantly different between the two strains. The results were only suggestive of an ATPase defect in audiogenic seizures.

When we reviewed experimental models for epilepsy, one of us (W.H.R.) observed that the histopathology of ouabain-induced epileptic foci was strikingly similar to vacuolizations observed in the late stages of some slow virus diseases (Kuru, Creutzfeldt-Jacob, Scrapie). We obtained brain tissue from infected animals (curtesy of Laboratory of Central Nervous System Studies, NINCDS) and measured ATPase activities in the diseased areas. Preliminary results indicated that in the affected areas enzyme activity was reduced by about 50 per cent. Current experiments deal with the problem of the specificity of this partial ATPase deletion.

Significance: We have predicted that ecto-ATPase and ecto-5'-nucleotidase would be nearly ubiquitous in eukaryotic cells; other investigators elsewhere are now making similar observations. We believe that our studies have initiated a new phase of plasma membrane research and that further investigations into the "sidedness" of membrane macromolecules will reveal new insights into the structure and function of the plasma membrane.

Our observations on the effects of ectopic ATP as an apparent mediator of membrane permeability have generated quite a bit of interest. We have speculated that this effect was related to the function of ecto-ATPases but it is probable that the enzyme(s) subserves other functions as well.

Our studies of the properties of adenosine deaminase in various cell cultures derived from the CNS will contribute to a better understanding of the role of the adenine nucleotides in the brain. No definite conclusions can be drawn as yet from the experiments with audiogenic seizure-prone mice.

Proposed Course: Further experiments are in progress which are designed to probe into the transverse asymmetry of the plasma membrane. It is planned to enlarge on current pilot studies to solubilize and characterize the ecto-enzymes, first in cell cultures of neural origin and eventually from brain tissue. We shall repeat the assays of ATPases in mice with audiogenic seizures; conditions will be chosen which will allow for partia purification of ecto-ATPases.

Key Words: plasma membrane, ecto-enzymes, ATPases, convulsive diseases, slow virus diseases.

Honors and Awards: None.

Publications:

1. Trams, E. G., Lauter, C. J., Koval, G. J., Ruzdijic, S., and Glisin, V.: Plasma Membrane Marker Enzymes in Developing Sea Urchin Embryos. Proc. Soc. Exp. Biol. Med. 147: 171-176, 1974.

2. Reichert, W. H., Lauter, C. J., and Trams, E. G.: Inorganic Pyrophosphatase in Cultured Cells. Biochim. Biophys. Acta 370: 556-563, 1974.

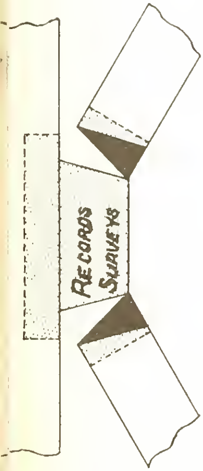
3. Trams, E. G.: Evidence for ATP Action of the Cell Surface Nature 252: 480-482, 1974.

HOW TO USE
THESE SEPARATORS

Use one page for each separation.

Select appropriate tab, add further identification if desired, and cover it with scotch tape.

Cut off and discard all tabs except the one covered by tape.



TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

ANNUAL REPORT
July 1, 1974 through June 30, 1975
Laboratory of Perinatal Physiology, IR
National Institute of Neurological and Communicative Disorders and Stroke

Ronald E. Myers, M.D., Ph.D., Chief

The effects of a combined sequential partial plus total asphyxia have been studied in rhesus monkey fetuses. Total asphyxia alone leads to injury in brain stem structures while severe partial asphyxia alone causes brain swelling and injury to hemispheral structures. However, when fetuses are exposed to an episode of partial asphyxia followed by total asphyxia, injury to the basal ganglia occur either alone or in combination with greater or lesser injury to brain stem structures on the one hand when total asphyxia predominates or with lesions affecting primarily other hemispheral structures on the other hand when partial asphyxia predominates. Thus, a spectrum of CNS injury occurs following combined sequential partial plus total asphyxia whose specific derivative patterns relate to the relative predominance of one or the other types of asphyxia. This spectrum of patterns of brain pathology in the experimental animal closely resembles a similar spectrum of pathology which is observed in perinatally injured humans.

Various anesthetic agents have been studied with respect to alterations which they induce in fetal oxygenation. Moderate pentobarbital anesthesia optimizes the oxygenation of the fetus whereas permitting the mother to come out of anesthesia on the operative table (light anesthesia) causes an asphyxia of the fetus presumably due to an increase in sympathetic tone. Pentobarbital given to the mother in heavy doses leads to pooling of her blood in the large visceral venous channels, the development of arterial hypotension, and a progressive severe asphyxia of the fetus. Thus, anesthesia when either too light or too deep may cause asphyxia of the fetus.

Studies have continued on the physiology of late decelerations of the fetal heart rate. Late decelerations occur in relation to uterine contractions during labor. The monitoring of the fetus with respect to late decelerations is of great significance with respect to the prevention of fetal asphyxia and perinatal brain damage. Fetal heart rate monitoring has found wide use in the United States during the last several years. However, considerable uncertainty still exists with respect to the relation between the appearance of late decelerations and asphyxia of the fetus. Our earlier studies have indicated a close correlation between the magnitudes of the various parameters of late decelerations and the oxygenation of the fetus. More recently, we have shown that the magnitude of late decelerations not only relates to the state of the fetus but also is affected by the physiologic state of the mother and particularly with respect to her blood pressure. The fetuses of hypotensive mothers may exhibit late decelerations of large size at a given level of asphyxia of the fetus whereas those of normotensive mothers may show only minimal or absent late decelerations. Collaborative studies with the Department of Obstetrics and Gynecology at the University of Alabama have indicated that the latency period of occurrence of late decelerations in the human fetus does indeed importantly reflect the absolute state of oxygenation of the fetus irrespective of blood pressure level of the mother and regardless of the magnitude of the late deceleration. Thus, though various parameters of late deceleration of the fetus may be affected by the physiologic state of the mother, the latency of late decelerations critically define the status of the fetus irrespective of status of the mother. Utilization of computer systems which respond to this important variable gives important information as to status of the fetus and serves as an important aid in the prevention of fetal brain damage.

Studies on the effects of severe hypoxia on the juvenile monkey have continued. The juvenile monkey may sustain severe degrees of hypoxia without altering its physiologic state. However, when the hypoxia extends beyond a certain severity, slowing of the heart and decrease in blood pressure appear. The more severe the hypoxia the greater the magnitude of these alterations in vital signs. Such vital signs changes begin when the pO_2 of the arterial blood of the animal approaches 22-25 mm Hg. At the same time that vital signs changes appear, the electroencephalogram develops slowing of its wave form and diminution in its amplitude. Complete EEG flattening appears when the pO_2 has diminished to 18-20 mm Hg, depending upon level of blood pressure. Just as the blood pressure level critically affects the behavior of the vital signs during hypoxia, so it determines outcome with respect to brain pathology. If the blood pressure is artificially maintained with pressor agents, the damaging effects of severe hypoxia may be greatly forestalled or prevented. Contrariwise, diminutions in blood pressure which are associated with diminished cerebral perfusion add to the effects of hypoxia and exacerbate the severity of brain injury.

Severe degrees of hypoxia lead to edema of the brain. This brain edema is associated with increases in intracranial pressure, increases in electrical impedance, and flattening of the EEG. Many animals which exhibit brain swelling die in the early hours of respiratory arrest with signs of compression of the brain stem. The few animals which survive which earlier exhibited evidences of severe brain swelling show patterns of pathology in the brain which are secondary to the swelling itself and include unilateral or bilateral injury to hippocampus, focal lesions in occipital lobe located in relation to the edge of the tentorium, and kissing lesions of the superior surface of cerebellum. If the severe hypoxia is combined with a brief period of anoxia (as occurs when blood pressure briefly drops to low levels and cerebral perfusion ceases) the pathology may affect the caudate nucleus, the putamen, and the globus pallidus. On the other hand, if the animals have been hyperventilated and the development of acidosis prevented, injury restricted to white matter may ensue.

Studies on hypotension have continued. Juvenile or adult animals may survive 30-minute episodes of hypotension where the blood pressure has been diminished with ganglionic blocking agents to levels as low as 30-40 mm Hg. At this blood pressure level, even though blood oxygen contents are well preserved, brain swelling develops and may lead to death of the animal during the second to fourth hour following restoration of blood pressure. The brain swelling, again, is associated with EEG flattening, increases in cerebral impedance, and signs of brain stem compression and respiratory arrest. Other animals may survive such episodes of hypotension and show no brain injury and develop no brain swelling. During the periods of hypotension themselves, increases in cortical impedance, flattening of the EEG, and decreases in frequency of the wave form are seen as the cerebral blood flow decreases below critical levels.

Changes in potassium distribution within the various compartments of the brain have been studied during hypoxia and anoxia. The normal potassium concentration of the cortical subarachnoid fluid by direct determination using atomic absorption spectrophotometry is 2.6 ± 0.1 mEq/l. The threshold value of the partial pressure of oxygen in the arterial blood at which the potassium concentration increases is 28-30 mm Hg. Exposure of animals to such severe hypoxia also depresses cardiovascular performance and causes lowering of blood pressure. If the blood pressure is maintained above 80-100 mm Hg by the use of pressor agents, the magnitude of the alteration of K^+ concentration of the cortical subarachnoid fluid remains less than 5-8 mEq/l irrespective of the magnitude of the depression of the oxygen pressures in the arterial blood. However, if the blood pressure decreases below 40-50 mm Hg, the K^+ concentration in the cortical subarachnoid fluid rises to values which exceed 15 mEq/l. The cisterna magna fluid reflects the changes in K^+ concentration of the extracellular fluid of the brain only poorly and after long delay compared to the cortical subarachnoid fluid.

Studies with potassium selective microelectrodes have enlarged our understanding of the behavior of potassium during dysergic states. The potassium ion behaves quite differently according to whether oxygen deprivation is partial or total i.e., whether the animals are subjected to hypoxia or anoxia. During hypoxia, the increases in potassium ion activity in the cortical extracellular fluid generally remains below 12-15 mEq/l. The severity of hypoxia required to lead to changes in potassium activity measured using ion-selective microelectrodes (20-22 mm Hg) was greater than that required to cause changes in potassium concentration in cortical subarachnoid fluid as measured using the atomic absorption spectrometer. On the other hand, when the animals were subjected to anoxia (were breathed with nitrogen or the trachea occluded) the potassium concentration in the cortical extracellular fluid increased rapidly (within several minutes) to levels as high as 60-80 mEq/l. These changes in potassium activity were associated with EEG flattening and a rapid negative shift in the DC potential. In these respects, the behavior of the cortical tissue under anoxic circumstances is closely similar to that which has been described with respect to spreading depression. An analysis is underway of the characteristics of the rising and falling phases of the changes in potassium concentration during exposure to hypoxia and anoxia. Preliminary analysis suggests that the increase in potassium concentration (its rise-time) is a complex function and cannot be described either as straight line or logarithmic. On the other hand, the decline in potassium concentration is logarithmic and the slope of the decline when plotted on semilog scales is related to the duration of the preceding period of anoxia itself - slower declines characterize longer durations of anoxia.

Juvenile rhesus monkeys overtrained on two visual discrimination tasks were subjected to 14-minute episodes of cardiac arrest induced by injections of potassium chloride. These animals were neurologically depressed for 6-12 hours following resuscitation. They failed to react to visual stimuli at a time when apparently normal responses could be elicited to tactual or auditory stimuli. When replaced in the visual discrimination apparatus prior to about 12 hours, the animals regularly sat in a corner and failed to respond. However, after about 18-20 hours, they began to respond but hesitatingly and with long intertrial intervals. When they did resume responding level of performance was uniformly high even though the rates of responding were depressed. Within several days, however, both the rates as well as the levels of responding returned to normal. All animals which could be resuscitated (6 out of 9) showed no neurologic deficits although lesions were later demonstrated at post mortem examination in several of the cranial nerve nuclei (including the IIIrd, IVth and VIth). These behavioral studies confirm earlier pathologic studies which demonstrated minimal pathologic changes in animals subjected to episodes of circulatory arrest lasting less than 12-14 minutes.

Serial No. Z01 NS 01388-10 LPP
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Perinatal asphyxia and its CNS consequences

Previous Serial Number: Same

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators:
Myron D. Ginsberg, M.D.
Karlis Adamsons, M.D.
Theodore Kerenyi
Salvatore Condorelli, M.D.
Peter Symchych
Arsenio Comas
Alfred Brann, M.D.
Jacque F. Roux
Ermelando V. Cosmi, M.D.
Eberhardt Mueller-Heubach
Loretta Strauss
Juan Figueroa-Longo

Cooperating Units:
Mt. Sinai School of Medicine, New York
University of Pennsylvania, Philadelphia
University of Rome, Rome, Italy
College of Physicians and Surgeons, New York
University of Puerto Rico, San Juan
University of Montreal, Montreal, Canada

Man Years:

Total	1.17
Professional	.98
Other	.19

Project Description:

Objectives: To determine the clinical and neuropathologic changes produced by asphyxia of the fetus or newborn and to correlate the patterns of neuropathologic change with functional alterations observed at the time of injury.

Methods Employed: Monkey fetuses are delivered by Cesarean section and subjected to episodes of total asphyxia (anoxia) for predetermined lengths of time by placing a rubber sac over the fetal head and clamping the umbilical cord. In some instances, an effort is made to produce the maximum injury compatible with survival. Other fetuses are subjected to episodes of partial asphyxia (hypoxia) which may be produced in a variety of ways most of which are associated with an impaired intervillous space perfusion. The cardiovascular, acid-base, respiratory gas, and respiratory changes which occur during and after the asphyxia are recorded and later correlated with the clinical and brain pathologic abnormalities produced.

Major Findings: With the varying durations of asphyxia, varying severities of brain damage are produced. The most common clinical alteration observed following anoxia is a widespread sensory loss manifested as abnormal posturing and placing reactions, altered sucking and swallowing, altered vocalization, and varying degrees of ataxia and motor weakness. With more severe damage, the animals show changes in postural tone and in use of the extremities. Total paralysis may be seen and the extremities may show a severe flexor or extensor hypertonus. Pathologically, destructive changes affect various brain stem loci and the thalamus. Damage to cortex or basal ganglia rarely occurs with anoxia.

Episodes of partial asphyxia (hypoxia) do not damage lower brain stem structures, but, instead, affect various hemispheral structures including the cortex, the hemispheral white matter, and the basal ganglia. These hemispheral patterns of brain injury closely resemble the patterns of injury observed after human perinatal asphyxia.

Significance: The intent is to reproduce the neuropathology of human cerebral palsy and mental retardation. Ulegyria, atrophic cortical sclerosis, lobar atrophy, status marmoratus, periventricular leukomalacia, porencephaly, hydranencephaly, and microcephaly have all been experimentally reproduced in a controlled fashion.

Proposed Course of Project: We will attempt to determine the critical parameters which lead to a deterioration of the vital functions of the fetus during asphyxia and to relate these physiologic alterations to the patterns of brain injury produced. Efforts are under way to develop techniques to prevent the deterioration of the fetus when asphyxiated and to prevent or postpone the occurrence of perinatal brain injury.

Honors and Awards: None

Publications:

Cosmi, E.V., Condorelli, S., and Myers, R.E.: Rianimazione del neonato di pecora dopo asfissia acuta endouterina. Acta Anaesth. Ital. 24/2: 133-146, 1973.

Mueller-Heubach, E., Myers, R.E., and Adamsons, K.: Maternal factors affecting fetal condition during labor and delivery. Ob/Gyn Digest. 16: 11-18, 1974.

Ginsberg, M.D., and Myers, R.E.: Fetal brain damage following maternal carbon monoxide intoxication: An experimental study. Acta Obstet. Gynec. Scand. 53: 309-317, 1974.

Myers, R.E., Symchych, P., Strauss, L., Comas, A., Figueroa-Longo, J., Kerenyi, T., and Adamsons, K.: Morphologic changes of uterine wall following intraamniotic injection of hypertonic saline in rhesus monkey. Am. J. Obstet. Gynec. 119: 878-888, 1974.

Roux, J.F., and Myers, R.E.: In vitro metabolism of palmitic acid and glucose in developing tissue of rhesus monkey. Amer. J. Obstet. Gynec. 118: 385-392, 1974.

Myers, R.E.: Four patterns of perinatal brain damage and their conditions of occurrence in primates. In: Meldrum, B.S. and C.D. Marsden, (Eds): "Advances in Neurology", Vol 10 (Primate Models of Neurological Disorders). Raven Press, 1975, New York, pp. 223-234.

Bran, A.W., Jr., and Myers, R.E.: Central nervous system findings in the newborn monkey following severe in utero partial asphyxia. Neurol. 25: 327-338, 1975.

Myers, R.E.: Maternal psychological stress and fetal asphyxia: A study in monkey. Am. J. Obstet. Gynec. 121: 47-63, 1975.

Serial No. Z01 NS 01464-09 LPP
1. Laboratory of Perinatal Physiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biochemistry of the recovering rhesus monkey from various different states of energy deprivation

Previous Serial Number: Same

Principal Investigator: Michio Yamaguchi, M.D.

Other Investigators: Ronald E. Myers, M.D., Ph.D.
Americo Rivera, Jr., Ph.D.
James R. Miller, M.D.

Cooperating Units: Rockefeller University, New York

Man Years:

Total:	2.8
Professional:	1.8
Other:	1.0

Project Description:

Objectives: To define and compare the biochemical changes induced in the brain by hypoxia and anoxia and to relate these changes to the development of brain edema.

Methods Employed: Monkeys under barbiturate anesthesia are subjected to episodes of anoxia or hypoxia. The blood pO_2, pCO_2 and pH as well as blood pressure, respiratory rate, and EEG were recorded. Tissue samples were then taken from the brain and frozen in liquid nitrogen. Other samples were chilled and homogenized with isotonic sucrose solution or deionized water. Those homogenates were used for determination of enzyme activities (Na, K-stimulated ATPase, Arylsulfatase A, Acid and Alkaline Phosphatases). Other brain samples were analyzed for water and electrolyte contents. Quick-frozen samples were stored at -80°C and assayed for ATP and cyclic AMP contents.

Major Findings: While both anoxia and hypoxia cause reductions of brain ATP content, that which followed anoxia was the greater. Cyclic AMP content was slightly but significantly increased but only in the brains of animals exposed to anoxia. Among enzyme activities studied only alkaline phosphatase activity was increased in the brains of animals exposed to hypoxia or anoxia.

The water contents of cerebral cortex, caudate nucleus, and white matter were determined by the method of Elliot and Jasper. Neither anoxia nor hypoxia caused significant changes in the water content of brain samples when sampled at the termination of the episodes.

Significance: The greater derangement in the metabolic state of the brain is produced by anoxia where the ATP level is reduced to low levels. At the same time minimal changes are demonstrated with respect to the activities of a variety of enzymes. The deficits following these oxygen deficiency states seem most directly related to altered energy metabolism rather than altered enzyme systems.

Proposed Course of Project: Thus far our analyses have been restricted to the early stages of exposure to oxygen deficiency. However, brain swelling develops only late following hypoxic exposure. Thus, our analyses will be extended to animals following resuscitation from hypoxia during that phase when brain swelling develops to ascertain the behavior of the various parameters measured above. We also will analyze the beta-Glucuronidase activity, lactate contents, and the integrity of blood brain barrier in these animals.

Honors and Awards: None

Publications:

Rivera, A., Jr., Miller, J.R., and Martinez de Jesus, J.: Glycogen of the brain and vital organs of monkeys recovering from systemic ischemia. Amer. J. Physiol. 225: 982-985, 1973.

Rivera, A., and Martinez de Jesus, J.: Starvation and the glycogen of the brain and vital organs of the rhesus monkey. J. Nutr. 104: 1189-1194, 1974.

Serial No. Z01 NS 01699-07
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Functions of forebrain commissures in learning

Previous Serial Number: Same

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators: Kazuo Hara, Ph.D.
Shun-ichi Yamaguchi, Ph.D.

Cooperating Units: Department of Psychology, International Christian University, Mitaka, Tokyo, Japan

Project discontinued.

Serial No. Z01 NS 02020-03 LPP
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Pathophysiology of brain injury produced by cardiovascular and pulmonary disorders

Previous Serial Number: Same

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators: William F. Blank, M.D.
Howard S. Kirshner, M.D.
Gary S. Kopf, M.D.
David M. Mirvis, M.D.
Francis W. Gamache, M.D.

Cooperating Units: None

Man Years:

Total:	4.06
Professional:	3.14
Other:	.92

Project Description:

Objectives: To develop animal experimental models of human brain disease produced by a variety of cardiovascular and pulmonary disorders. These include studies of interferences with oxygen availability to the tissues either due to cessation of circulation (cardiac arrest), hypotension, pulmonary disease, myocardial abnormalities, or intoxications with such agents as carbon monoxide or cyanide.

Methods Employed: Juvenile or adult monkeys are anesthetized with pentobarbital and one femoral artery and vein catheterized for the recording of blood pressure, heart rate, and for withdrawal of blood samples for analysis of acid-base and respiratory gas state. Electrocardiographic and electroencephalographic leads are placed and a variety of physiologic parameters monitored including cerebral impedance, intracranial pressure, electrolyte composition of blood and CSF, EEG, glucose content of blood and CSF, etc. The animals are subjected to one of the variety of insults described above and measures are taken of the physiologic and biochemical changes produced. The animals are resuscitated and observed both neurologically during survival and pathologically following sacrifice.

Major Findings: The neuropathology of carefully regulated episodes of circulatory arrest affects structures in the brain stem. Hemispheral structures including the cerebral cortex, hemispheral white matter, and basal ganglia remain unaffected. These findings are contrary to those described in the human and the present work has made it clear that the human patterns of injury following cardiac arrest are due not to the stasis of blood flow but to hypoxia which is incurred during efforts at resuscitation.

The extent of blood pressure lowering which the monkey can sustain without evidence of brain injury is in the order of 30 mm Hg arterial blood pressure. Animals subjected to blood pressures lower than this may die during the period of hypotension due to cardiac arrhythmias or failure; may die in the early hours following blood pressure restoration of cardiogenic shock; or may die during the first 4-48 hours with severe brain swelling and brain stem compression. Only a small proportion of the animals subjected to severe hypotension survive to show static, long-term lesions.

Twelve minutes of cardiac arrest can be sustained at normal body temperatures without evidence of brain injury. Thereafter, loci in the brain stem show injury--the more prolonged the stasis the more widespread the injury. After 20 minutes of stasis few animals survive due to development of cardiogenic shock.

Hypoglycemia is associated with minor alterations in respiratory gas and acid-base state but leads to significant hypotension and circulatory impairment. When hypoglycemic animals are artificially respired, the patterns of pathology observed affect the basal ganglia, the cortex and the hippocampus. The long-term changes consist of convolutional atrophy and dementia. Hypoglycemia does not, by itself, lead to brain swelling.

Significance: These studies attempting to elucidate the pathyphysiology and the pathogenesis of human patterns of brain injury caused by cardiovascular or pulmonary diseases, are important to enable us to develop techniques for their prevention or amelioration.

Proposed Course of Project: These studies will be continued to attempt further clarification of the pathophysiology of these various patterns of pathology caused by respiratory and circulatory disorders.

Honors and Awards: None

Publications:

Myers, R.E.: Neuropathology of total oxygen lack (anoxia) in rhesus monkey. In: Cervos-Nararro, J., ed., Pathology of Cerebral Microcirculation. Walter de Gruyter, Berlin, 1974, pp. 299-306.

Kopf, G.S., Mirvis, D.M., and Myers, R.E.: Central nervous system tolerance to cardiac arrest during profound hypothermia. J. Surg. Res. 18: 29-34, 1975.

Gamache, F.W., and Myers, R.E.: Effects of hypotension on rhesus monkeys. Arch. Neurol. 32:372-380, 1975.

Serial No. Z01 NS 02021-03 LPP
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Short- and long-term behavioral deficits produced by dysergic brain disease

Previous Serial Number: Same

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators: Shun-ichi Yamaguchi, Ph.D.

Cooperating Units: None

Man Years:

Total	1.65
Professional:	.48
Other	1.17

Project Description:

Objectives: To determine the short- and long-term effects on perceptual learning and memory function of a variety of brain insults produced by cardiovascular or pulmonary diseases and/or states of intoxication.

Methods Employed: Juvenile rhesus monkeys are trained on visual pattern discrimination tasks to choose one and to avoid the other of two visual stimuli. Banana pellet food reward is used to reinforce the correct pattern of responding. The monkeys are then subjected to episodes of energy deprivation (circulatory arrest, severe hypotension, severe hypoxia, total asphyxia, carbon monoxide intoxication, etc.). The animals are then resuscitated and again tested on the behavioral response. If response deficits are defined, the time course of restoration of performance is then determined. Permanent deficits are defined and quantitated.

Major Findings: Up to 14 minutes of circulatory arrest can be sustained with no effects on visual memory or discrimination.

Significance: The neurologic assessment of the clinical state of the rhesus monkey is difficult and leads only to crude measures of neurologic state. Utilization of more sophisticated behavioral test methods permit the assessment of subtle changes in overall nervous system activity, both with respect to temporary reversible disturbances and also to permanent deficits related to specific lesions.

Proposed Course of Project: To extend these test methods to assessment of the consequences of other pathologic states.

Honors and Awards: None

Publications: None

Serial No. Z01 NS 02022-03 LPP
1. Laboratory of Perinatal Physiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Neural mechanisms underlying social behavior and emotion in the rhesus monkey

Previous Serial Number: Same

Principal Investigator: Shun-ichi Yamaguchi, Ph.D.

Other Investigators: Ronald E. Myers, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total:	1.88
Professional:	.48
Other:	1.40

Project Description:

Objectives: To investigate the neural mechanisms underlying social behavior and emotion in rhesus monkey.

Methods Employed: A variety of techniques are used to study social behavior and emotion in the sub-human primate. In some instances, monkeys are placed in observation cages and their spontaneous patterns of vocalization are quantitated. In other instances, the general cage behavior of the animals and their reactions to challenge by the experimentor are defined. In other instances, the animals are placed in gang cages as small social groups and their social interactions determined.

Major Findings: Lesions of cortical areas related to speech in humans failed to alter the vocalization patterns of the rhesus monkey. On the other hand, areas of cortex having to do with the regulation and control of social behavior, including the prefrontal and anterior temporal cortex, produce major decreases or total abolition of vocal response. Bilateral lesions of subcortical structures revealed that amygdala and tegmentum of midbrain, both centers important to the regulation of social behavior, also alter the patterns of vocal response. Gang cage studies have demonstrated major deficits in all aspects of social behavior with lesions of prefrontal and anterior temporal cortex. Cingulate cortex lesions, on the other hand, produce only equivocal changes. Other studies with split-brain animals (destroyed crossing retinal fibers at optic chiasma) have shown that unilateral prefrontal and/or anterior temporal cortex lesions produce bilateral deficits as tested over the two sides of the body and in the two half-fields but the deficit is much greater in the half-field ipsilateral to the hemisphere of lesion. When corresponding lesions are added to second hemisphere, additional bilateral defects are demonstrable. Thus, elements exist both for laterality and bilaterality of these functions with respect to their cortical localization.

Significance: These studies which attempt to identify brain structures involved in the regulation and control of social behavior are of major importance in our efforts to understand the biologic foundations of disorders of behavior.

Proposed Course of Project: Further examination of brain stem structures for their contribution to expression of social behavior and affect.

Honors and Awards: None

Publications: None

Serial No. Z01 NS 02081-02 LPP
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Experimental production of cerebral malformations by use of teratogenic agents or severe asphyxia during pregnancy

Previous Serial Number: Same

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:	
Total	.94
Professional:	.14
Other:	.80

Project Description:

Objectives: To produce cerebral malformations in the developing rhesus monkey and sheep fetus by treating the mother with teratogenic agents or subjecting her to severe asphyxia at different stages of pregnancy. To carefully study the morbid anatomy of the cerebral malformations induced and to relate them to the time of pregnancy during which the insulting agent was administered. The patterns of induced morphologic abnormalities in the experimental animal will be compared with the known patterns of cerebral malformation in the human.

Methods Employed: Pregnant rhesus monkeys and sheep whose pregnancy lengths are known are injected with MAM-acetate (methylazoxymethonal acetate, 10 mg/kg). Alternatively, the mothers are subjected to a single episode of severe asphyxia. Later, the mothers are anesthetized and the fetuses delivered. The body and organ weights of the newborn are determined and the brain examined grossly and microscopically. The patterns of malformation induced are related to time of gestation during which the insulting agent acted and the patterns of cerebral malformation are determined in relation to the development of anlaga tissue at the time of insult. The induced cerebral malformations are compared and contrasted with known patterns of malformation in the human.

Major Findings: The toxic dose of MAM-acetate was defined for rhesus monkey and the first monkeys were injected. Similarly, the severity of asphyxia required to produce brain injury of the developing fetus has been defined and efforts are made to subject fetuses to severe asphyxia during earlier gestational ages. Unexpectedly, single doses of MAM-acetate at toxic levels failed to reduce cerebral malformations in the animals tested thus far.

Significance: The definition of the timing and of the inciting agents which lead to cerebral malformation in the human is poorly understood. Thus, the above study should provide insights into the pathogenesis and morphogenesis of cerebral malformations as they affect the human.

Proposed Course of Project: Injection of animals, harvesting of fetuses, weighing of internal organs, and examination of brains. Correlation with human findings.

Honors and Awards: None

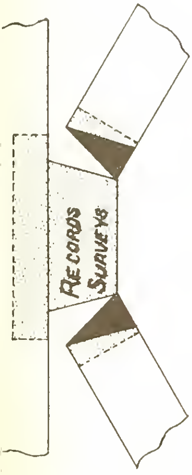
Publications: None

HOW TO USE
THESE SEPARATORS

Use one page for each separation.

Select appropriate tab, add further identification if desired, and cover it with scotch tape.

Cut off and discard all tabs except the one covered by tape.



TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

ANNUAL REPORT
July 1, 1974 through June 30, 1975
Laboratory of Molecular Biology
National Institute of Neurological and Communicative
Diseases and Stroke
Intramural Research

Ernst Freese, Chief

The Laboratory has investigated problems of cell differentiation, cellular effects of lipophilic acids, and properties of autointerfering defective viruses. All investigations employed microorganisms or mammalian cell cultures, because it was important for biochemical studies to have large numbers of identical cells. 1) The investigation of differentiation has so far been limited to bacilli in which many different auxotrophic and cacogenic mutants have been isolated and analyzed. It was found that larger amounts of certain vitamins and other compounds are required for optimal differentiation than for maximal growth, that glycerol-P supply has to be precisely controlled, and that high intracellular concentrations of the phosphates interfere with differentiation. Commitment was shown to depend on the environmental conditions under which it is tested; it is not due to a single trigger reaction. These results are expected to have general significance for all differentiating organisms. 2) Lipophilic acids, which include most food additives and antiseptics as well as a large number of known teratogens, inhibited the growth of both bacteria and mammalian cell cultures with the same potency for most investigated compounds. The correlation of the inhibitory potency with the lipophilic partition coefficient indicates how food additives and antiseptics that are relatively harmless to man can be selected, and how compounds or drugs that have the potential to cause brain damage or teratogenic affects can be avoided. Some of the low molecular weight fatty acids were unexpectedly potent inhibitors of HeLa cell growth, caused shape changes and induced a sialyltransferase activity indicating partial reversion of the cancerous to a more normal cell type. 3) The molecular properties of defective RNA viruses were studied with the aim to understand why these viruses, which can grow only in the presence of a normal virus, outgrow the normal viruses. Single and double stranded defective viruses were observed that contain different, non-overlapping portions of the virus genome. This suggests that artificial RNA sequences may also work as interfering RNA's. These investigations are important because interference may be used to cause the abortion of a virus infection.

1. Effect of deficiency or excess of metabolites on differentiation. When different auxotrophic mutants of Bacillus subtilis were grown in media containing different concentrations of the required compound, it was observed that for certain vitamins and other compounds much higher concentrations were required for optimal differentiation than for maximal growth. In particular, five times the amount of nicotinic acid (or nicotinamide) and twenty times the amount of thiamine were needed for optimal sporulation, which represents differentiation in this microbial system. These findings support the statements made by Williams and others who have claimed that

the amounts of certain vitamins required during human embryogenesis, childhood development or tissue regeneration are higher than those needed for the maintenance of adult life. The high developmental requirements for these vitamins indicate that some enzymes for which the vitamins provide the necessary coenzymes have to be especially active during differentiation. For example, the coenzyme derived from thiamine is thiamine-PP which is needed for relatively few enzymes in the cell. For the enzymes requiring thiamine-PP as co-factor, we have shown that the pyruvate dehydrogenase system is most important with respect to sporulation, because the thiamine requirement can be greatly reduced by the addition of acetate to the medium. The importance of this enzyme system for sporulation has also been demonstrated directly in a mutant lacking the activity of the pyruvate dehydrogenase system; again sporulation is possible only when the cells are supplied with high concentrations (70 mM) of acetate.

Whereas, the differentiation of the previous auxotrophic mutants was optimal when the required compound was added in excess of the minimally required concentration, sporulation of a glycerol-requiring mutant required a much more precise control of the necessary compound. The addition of more glycerol to the medium did not restore sporulation. The reason is that glycerol can be rapidly metabolized into compounds suppressing sporulation so that the differentiation process does not start until glycerol has been used up. At that time, however, the cell membrane becomes thin and eventually ruptures, as was shown by the release of ATP and the collapse of the membrane away from the cell wall as seen in the electron microscope. However, an ideal way of completely curing the differentiation capability of this mutant was to add excess of α-glycerol-P to the medium from the beginning. This compound is taken up at such a slow rate, that it can not suppress differentiation of bacilli, and yet it can be taken up at a rate that suffices to allow a normal membrane synthesis throughout growth and development. In the presence of glycerol-P, the glycerol mutant sporulates therefore as well as the standard strain. This remarkable finding could be extended to a mutant deficient in P-enolpyruvate carboxykinase, the first enzyme of gluconeogenesis. Again, this mutant can not sporulate, or even develop the typical asymmetric prespore septa but sporulation is restored by the addition of glycerol-P to the medium. The possible consequences of these findings to hereditary diseases should be apparent.

Some compounds prevent differentiation when their intracellular concentration is too high. To identify the metabolites causing this effect, single, double, or triple mutants were isolated in which the accumulation of one compound could be controlled and its further metabolism was prevented. In this way, it was shown that the accumulation of glucose-6-P, fructose-6-P, or glycerol-P each prevented sporulation which could occur normally when the accumulation was avoided. Sporulation was blocked before the cells had developed any asymmetric prespore septa. Similar to the situation of hereditary diseases, such as galactosemia or fructose maple-sugar disease, the problems could be avoided by leaving out the nutrient component that would give rise to the accumulated intermediate; normal differentiation resulted. In the glycerol-P accumulating mutant another approach could be used which appears generally promising; Instead of carefully controlling the supply of glycerol, which is obviously difficult to manage, the addition of malate to the medium also resulted in norami sporulation, because malate partially represented glycerol kinase.

2. Commitment to differentiation. A phenomenon that is typical to differentiation in all organisms is that of commitment. It consists in the ability of a differentiating cell to continue differentiation even when the cell is exposed to conditions under which it would otherwise grow and not differentiate. The existence of this commitment can be extablished in several ways including the transplantation of nuclei or the transfer of differentiating cells to a new environment. This phenomenon was believed to demonstrate the existence of a single type of molecular change in the cell, a trigger, which was supposed to cause the commitment. The "trigger" idea was responsible for the ready acceptance of claims that an observed alteration of a single molecular species represented the initiating event of differentiation. Such proposed triggers have included the appearance of an antibiotic or protease activity, a change of RNA polymerase, etc. The Laboratory has now conclusively shown that the cellular stage at which cells are committed to continue their differentiation process when exposed to a new environment depends on both the environment in which these cells originally were at the time of transfer and on the new environment. Thus, the medium in which bacilli are grown as well as the medium to which they are transfered determine the time at which commitment can be observed. In particular, commitment of Bacillus megaterium with respect to transfer to a minimal sporulation medium containing aspartate or glutamate occurs before the commitment with respect to transfer to glycerol, glucose, sucrose, and that occurs earlier than the commitment with respect to a rich medium. Correlations in the electron microscope have shown that the earliest commitment can occur before any sporulation specific membrane changes have occurred whereas, the latest commitment with respect to the richest medium occurs when the forespore double-membrane has been closed. These events have been correlated with membrane changes: The commitment with respect to glucose or sucrose can be related to a simultaneous decrease in the transport mechanisms of these compounds, whereas the latest commitment can be associated with the fact that the forespore double-membrane can no longer transport substrates actively. Therefore, a competition sets in between the attempt of the forespore cell to grow and the attempt of the whole cell to continue the differentiation process. Consequently, the spore develops to a larger size when these cells are transferred to a fresh medium with respect to which they are commited than when they remain in the original medium.

At about the time at which the amino acid uptake of bacilli decreases, the motility of the cells also vanishes, although the concentration of ATP is maintained. Since both amino acid transport and motility are apparently energized by a proton gradient that in turn can be generated either by the electron transport system or by ATP, the decrease of these properties indicates an uncoupling from or a destruction of the proton gradient at this time of differentiation. Since this process has certain similarities with the differentiation in higher organisms, e.g. of tadpoles into frogs which is initiated by the uncoupler thyroxin, the properties of this uncoupling process are now under investigation.

3. Growth inhibition of bacterial and mammalian cells by lipophilic acids. Lipophilic acids, i.e., lipophilic compounds with acidic groups such as carboxyl or phenolic groups, are widely used by society for a variety of purposes. They comprise the majority of antimicrobial food additives, other preservatives, and antiseptics, and they include a large number of drugs. The lipophilic character allows these compounds to attach to and enter cellular membranes while the acidic moiety causes the transport of protons through cell membranes, a property which is either desired (preservatives) or an unwanted side-effect (e.g., for aspirin) of the compound. In bacteria, the ability of the lipophilic acid to equilibrate a proton gradient causes the destruction of bacterial transport of amino acids and keto acids and thereby inhibits bacterial growth. The addition of glucose or other carbohydrates, whose transport is not energized by the proton gradient, restores substrate and ATP synthesis and thus restores growth. The same compounds also inhibit the growth of mammalian cells mostly with the same potency but for some compounds with a much higher potency than observed for bacterial inhibition. Generally, the potency of the compound increases with its lipophilic tendency, i.e., with the partition coefficient measured, for example, between octanol and water. Only lipophilic acids with small molecular weight and low lipophilicity are acceptable as food additives since they do not taste badly. Apparently, animals have developed an aversion against the more dangerous compounds with longer aliphatic chains or otherwise high lipophilicity to which they were exposed in nature. Thus, only acetate or propionate are acceptable among the saturated fatty acids, and only crotonate and sorbate among the unsaturated ones. However, no such protection is available for antiseptics or drugs for which compounds with very high lipophilicity have been developed. One such compound, hexachlorophene has been shown to cause brain damage in children and a large number of drugs have been identified as teratogens in mammalian tests. These compounds are now under investigation. Hexachlorophene is so lipophilic that once it has attached to a bacterium it no longer detaches and therefore, acts in an irreversible fashion. Our findings promise to provide an understanding for the different effectiveness of certain antiseptics and drugs in different environments and also for the specificity of these compounds toward certain mammalian cells. In order to investigate this specificity in more detail the effects of different teratogens on a variety of mammalian cell lines will be examined.

During the measurement of growth inhibition of HeLa cells it was found that propionate, butyrate, and pentanoate inhibited growth with a much higher potency than would have been expected from their partition coefficient. These compounds also caused, quite specifically, the production of long cell processes observed on Petri plates, a development which could be prevented by Colcemid or by a calcium ionophore. Apparently, these short-chain fatty acids cause microtubules to assemble and, since cell growth is inhibited, to prevent the usual disassembly observed during cell division. The process requires protein synthesis since it is inhibited by cycloheximide but once the required protein has been made cells will expand in the presence of cycloheximide even after they have been removed from the Petri plate and replated. Thus, preparation for cell division apparently impilies the formation of an inhibitor of microtubular assembly. The same cell expansion has been observed with neuroblastoma cells in the presence of serum, a process which is quite distinct from the much more rapid formation of thin cell processes which is observed in the absence of serum and which does not require protein synthesis. However, butyrate did not induce these shape changes in Chinese Hamster-Ovary cells which could specifically cause such changes with butyryl cAMP. Since these shape changes resemble the conversion of cancer cells to a more normal cell type, the sphingolipid pattern of HeLa cells was investigated in collaboration with the Developmental and Metabolic Neurological Branch, NINCDS. It was shown that the three small molecular weight fatty acids specifically cause an increase in the cellular sialyl-lactosyl-ceramide content of the cell membrane owing to an up-to fifteen-fold elevation of the CMP-sialic: Lactosyl-ceramide-sialyltransferase activity. So far, the induction of this enzyme and the formation of the shape changes were always correlated, the enzyme induction preceeding the shape changes by several hours. The effects of small molecular weight fatty acids on the growth of neoplastic cells in animals are now investigated in the Cancer Institute. We are analyzing the reason why butyrate causes these drastic cell changes and we are attempting to determine why CHO and HeLa cells respond differently to butyryl-cAMP and butyrate.

4. Properties of normal and defective vesicular stomatitis viruses. The Laboratory investigates the molecular biology of vesicular stomatitis viruses as a prototype of myxo-, paramyxo-rhabdo viruses, which are membrane enveloped RNA viruses responsible for a large number of diseases such as influenza, rabies, mumps, and measles. It is now clear that the virus RNA itself cannot be used as messenger RNA but has to be first transcribed by the RNA polymerase contained in the virus particles. Apart from this and other enzymes the major virus protein is the N protein with which the viral RNA is encased in the virus core. In collaboration with the Laboratory of Biology of the National Cancer Institute, it has been shown that the virus emerges from the cell in a bacillar form completely enwrapped into cellular membrane. Only due to artifacts of the preparation is the more usually observed bullet-shaped form produced. Of particular interest to the Laboratory is the investigation of autointerference, i.e., the fact that a defective virus has a selective advantage when it co-infects a cell with a normal virus. Defective viruses can be easily separated from the normal viruses by density gradient centrifugation. Since they cannot multiply in cells without the presence of a normal virus, a virus infection can be effectively aborted. J. Holland, La Jolla, California, has already demonstrated that this phenomenon can be used to rescue virus infected animals from certain death. However, since the use of defective viruses always harbors the danger that new viruses with a different tissue or host specificity might be selected, a thorough understanding of the mechanisms of autointerference will be necessary before human vaccines of defective viruses should be prepared. Present work in the Laboratory has shown that a practically unlimited number of different defective virus particles can be produced. Each defective virus preparation contains a large number of identical particles which shows that the defective particles are produced by deletion mutations. All of these virus mutants contain only part of the viral RNA, but some contain in addition to this RNA the complementary messenger RNA, attached to it covalently. Since the length of the viral RNA and the complementary RNA piece seems to be identical, the defective particles may have derived by cuts of a replication intermediate suggesting that the covalent attachment of messenger RNA to the viral RNA is a necessary

component of virus replication. The mechanism by which the defective RNA viruses interfere with normal virus replication is now under investigation. Since the Laboratory has found that non-overlapping parts of the virus genome can each serve as interfering RNA particle, it is possible that no particular RNA sequence is required for interference. If that were so, any RNA, e.g., poly-A, encased into a virus core with the N protein, might be just as effective for interference as the ribonucleoprotein core of a defective virus If that should be the case, superinfection by a large number of such particles containing harmless RNA could be a very effective means to abort virus infection.

Project No. Z01 NS 01244-11 LMB
1. Lab. of Molecular Biology
2. Section on Developmental
   Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Control Mechanisms and Differentiation

Previous Serial Number: Same

Principal Investigator: Ernst Freese, Ph.D.

Other Investigators: Peter H. Cooney, Ph.D., Eric Eisenstadt, Ph.D.

Cooperating Units: None

Man Years:

Total:	2.9
Professional:	2.4
Other:	0.5

Project Description:

Objectives: The developmental processes leading to cellular differentiation begin when cells stop growing and end when a genetically defined differentiated cell has formed. Many essential regulatory phenomena involved in differentiation are undoubtedly similar micro- and higher organisms. Since micro-organisms can divide faster, mutants can be isolated, and large numbers of identical cells can be obtained for biochemical studies we are presently investigating differentiation in bacilli, with the aim of uncovering those phenomena that are likely to be of general significance for all differentiation. In particular, we have investigated the effects of deficiencies and excesses of vitamins and organic phosphosphates on sporulation, the phenomenon of commitment of differentiation, and the consequence of phospholipid alterations on septation and sporulation.

Methods Employed: The compounds able to support rapid growth of *Bacillus megaterium* were determined by growing the cells on pools of different amino acids. Commitment to sporulation was followed in *Bacillus megaterium* by counting the number of phase-bright particles 10 hours after the end of exponential growth and relating it to the number in the control culture. The ratio between the count observed in the experimental culture and that in the control culture represents the fraction of committed cells at a given time of development. A new mutant was isolated which is deficient in branched-chain fatty acid biosynthesis. It requires any one of the three branched fatty acid primers for growth.

Major Findings: 1. The importance of vitamins for differentiation. When auxotrophic mutants requiring certain vitamins or other metabolites were grown in a sporulation medium containing different amounts of these compounds the following observations were made: five times more nicotinic acid was required for optimal sporulation than for maximal growth and twenty times more thiamine. This indicates that certain enzymes are particularly important for sporulation. In order to determine one of these enzymes, the effect of thiamine which is converted to thiamine-PP inside the cell, was further investigated. It was found that the concentration of thiamine needed for sporulation could be reduced by a factor of 10 when the medium also contained acetate (5 mM), indicating the importance of pyruvate dehydrogenase for differentiation. In fact, acetyl CoA or its extracellular precursor acetate are required during differentiation for both ATP regeneration and membrane synthesis. The importance of pyruvate dehydrogenase for differentiation was directly shown in a mutant lacking this enzyme: the mutant could sporulate only when high concentrations of acetate (70 mM) were present in the medium. The role of pyruvate dehydrogenase therefore seems to be both to destroy accumulated pyruvate and to provide acetate as precursor of acetyl-CoA needed during differentiation. If these results can be generalized, it seems apparent that in all organisms differentiation will require higher concentrations of certain metabolites than either vegetatively growing cells or the resting cells of an adult.

We have also correlated our investigations on the suppression of sporulation by excess of organic phosphates with electron microscope studies. Whenever cells accumulate an organic phosphate, differentiation is blocked very early as no asymmetric septa can be found.

2. Commitment of sporulation. Commitment is a general phenomenon observed in differentiating cells. It describes the fact that a differentiating cell reaches a state at which it will continue the differentiation process even when it is returned to an environment in which it would ordinarily continue to grow and not start to differentiate. These observations had created the impression that a single molecular reaction was responsible for a trigger-like change in the cell so that a committed cell would continue to differentiate no matter what the nutritional environment might be to which it were exposed. Our results have clearly destroyed this picture. We have found that transfer of cells to different types of media produces commitment at different times; e.g., exposure of cells to aspartate or glutamate can no longer prevent the differentiation process at a time at which exposure of cells to glucose or sucrose still can. The commitment with respect to glucose or sucrose was found to be correlated with a decrease in the transport of these compounds. The correlation of commitment with respect to other compounds and some cellular change is still under investigation. The latest commitment was observed with respect to a rich medium and a correlation between these commitment times and electron microscope observations show that the closure

of the forespore double membrane is the very latest sporulation stage at which commitment can be observed. Since at that time active transport into the forespore cell is no longer possible, because the cell is surrounded by two membranes that transport actively in opposite directions, substrate can enter this forespore cell only very slowly (by facilitated transport) and the differentiation process can therefore proceed without interference. Nevertheless, compounds can slowly enter the forespore cell, because transfer of cells to a fresh medium gives rise to distinctly larger spores than when the cells continue to remain in a poor medium.

3. Sporulation and mutants deficient in membrane synthesis. The asymmetric septation observed at the beginning of sporulation is an interesting phenomenon whose origin is unknown. Since several reports have been made concerning the alteration of the proportion of different branched-chain fatty acids during growth and sporulation, we decided to settle whether any particular branched-chain fatty acid or the ratio of straight to branched-chain fatty acids play a role in the control of septum formation or generally of sporulation. We are using two branched-chain fatty mutants both of which are unable to grow without a branched-chain fatty acid precursor but which can sporulate if the proper precursors are available. Since one of these mutants is also blocked in pyruvate dehydrogenase the branched-chain fatty acid property will have to be isolated by transformation. The two mutants are different in that one mutant can grow only when branched-chain fatty acids are provided, while the other can also grow in the presence of valine or isoleucine (1 mM) but not on leucine. Gas chromatographic analysis has established that the branched-chain fatty acid content can be controlled qualitatively and quantitatively both by the availability of branched-chain primers and the relative amount of branched to straight-chain fatty acids. We are presently using this mutant to control the ratio of branched to straight-chain fatty acids and that of different branched-chain fatty acids at specific times during the differentiation process. Preliminary observations already indicate that a partial deficiency of branched-chain fatty acids allow cells to elongate but not to divide, suggesting that the division process may indeed require critical amounts of branched-chain fatty acids.

Significance to Biomedical Research and the Program of the Institute: It has often been claimed that more vitamins might be necessary for embryogenesis, adolescent development, or regeneration. Our studies in a cellular system indicate that such claims are reasonable and suggest that higher amounts of certain vitamins are needed for differentiation than for regular adult life. Since we have shown that commitment to differentiation in bacilli depends on the environmental conditions, i.e., is due to a sequential reaction and not a sudden trigger, it appears likely that the same is true for differentiation in higher organisms. This finding could be significant for the understanding of the effect of teratogenic compounds. The investigation of phospholipid alterations on membrane septation again should be of general nature with significance to higher organisms.

Proposed Course of Project: It will be analyzed whether the time at which commitment occurs with respect to different compounds is correlated to the decrease in their transport or whether an enzymatic ahcnge in the cell is responsible for this timing. It will also be determined whether the decrease of amino acid transport and observed reduction in flagella movement are strictly correlated and also whether both of these effects are due to an uncoupling phenomenon in the differentiating cells. The effect of mutants defective in phospholipid synthesis on cell septation and differentiation will be examined.

Keyword Descriptors:

Differentiation
Bacillus subtilis
Sporulation
Commitment
Vitamins

Honors and Awards: None

Publications:

Freese, E., Ichikawa, T., Oh, Y.K., Freese, E.B., and Prasad, C.: Deficiencies or excesses of metabolites interfering with differentiation (vitamin deficiency, glycerol-phosphate dehydrogenase/glucose-6-phosphate/glycerolphosphate). *Proc. Nat'l. Acad. Sci., U.S.A.* 71: 4188-4193, 1974.

Lin, J., and Prasad, C.: Selection of a mutant of *Bacillus subtilis* deficient in glucose-6-phosphate dehydrogenase and phosphoglucoisomerase. *J. Gen. Microbiol.*, 83: 419-421, 1974.

Cooney, P.H., Freese, E.B., and Freese, E.: Commitment of *Bacillus megaterium* to sporulation. Spores VI, in press.

Project No. Z01 NS 01886-05 LMB
1. Lab. of Molecular Biology
2. Section on Developmental
   Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Developmental Cytology

Previous Serial Number: Same

Principal Investigator: Elisabeth B. Freese, Ph.D.

Other Investigators: Yong K. Oh, Ph.D., Tsutomu Nishihara, Ph.D. (Visiting Fellow), Yasutaro Fugita, Ph.D. (Visiting Fellow)

Cooperating Units: None

Man Years:

Total: 4.3
Professional: 3.8
Other: 0.5

Project Description:

Objectives: Differentiation consists of a sequential phenomenon leading from vegetatively-growing cells to highly differentiated ones. Since the sequential development can be followed in a large number of almost synchronized cells under the electron microscope, one can statistically determine the stages at which different biochemical mutants are blocked in their development and also correlate the stage of development with the commitment to differentiation. In addition, one can observe in developmental mutants whether any abnormal cell wall or membrane phenomena take place which may provide insight into the biochemical deficiencies of these mutants.

Methods Employed: For the morphological analysis, ultra-thin sections were studied with the electron microscope, EM6B of AEI and later with a Philips Electron Microscope, EM201. The mutants were grown in different sporulation media and samples were withdrawn during the developmental period in order to determine statistically which fraction of these cells had developed a certain stage of sporulation. The cells were fixed by glutaraldehyde, stained with osmium teteroxide and uranyl acetate, dehydrated, embedded in Epon, and after sectioning with Reichart Microtome, OMU2, stained by lead-citrate. For each time point, approximately 500 cells that were longitudinally sectioned, were examined to see their asymmetric septation type. The mutants had been isolated previously and were grown in nutrient sporulation medium with or without added phosphate or manganese ions.

Major Findings: 1. Stages of development and commitment. When cells are grown in a sporulation medium they start their developmental processes at the end of exponential growth and then go through a series of stages, some of which can be identified by their typical membrane properties under the electron microscope. One can ask whether any of these stages are significant for the phenomenon of commitment which occurs at different times of development with respect to cell dilution into different media. This experiment was performed by growing the cells initially in a minimal sucrose sporulation medium or a nutrient sporulation medium, and then transferring the cells to a minimal sucrose medium, a nutrient sporulation medium or a nutrient sporulation medium containing extra casein hydrolysate or glucose. The membrane stages of development were further subdivided into II, IIIa, b, c, IV, etc. It was observed that transfer of cells to a minimal sucrose sporulation medium allowed the expression of commitment before any asymmetric septum had been formed, transfer to a nutrient sporulation medium allowed commitment at about the time at which the prespore septum had been formed and transfer to the richest medium delayed commitment up to the time at which the forespore had been completely surrounded by the double membrane. Thus, if one wants to talk about any final commitment of differentiation it is the time of this membrane completion rather than the time of any other cellular changes such as the formation of a protease or the alteration of an RNA polymerase. That this point of development should be particularly important is understandable because the two membranes transport substrates in opposite directions against each other and therefore could allow only facilitated but no longer active transport into the forespore cell. Thus, the further sporulation development could then proceed unimpeded irrespective of extracellular conditions.

2. Properties of a glycerol-requiring mutant. A glycerol-requiring mutant (61106), derived from B. subtilis grows in nutrient sporulation medium containing 100 mM additional potassium-phosphate to a lower A_{600} (1.1) than without the phosphate addition ($A_{600}=2$). At the end of growth it releases ATP into the phosphate medium whereas it does not release ATP into a medium containing no extra phosphate. Under the electron microscope the ATP release is correlated with a severe collapse of the cytoplasmic membrane, separated from the cell wall. Much less change is seen without the extra phosphate. In the presence of phosphate, free fatty acids accumulate earlier and a more drastic change in the membrane phospholipid composition; i.e., the phosphatidylglycerol/phosphatidylethanolamine ratio is decreased. Apparently, the presence of phosphate causes a more rapid utilization of glycerol so that the cells run out of glycerolphosphate and thus phosphatidylglycerol at the time which they still have all other cell components available for growth. Consequently, the protein synthesis continues, the cells enlarge and evenually rupture. The cell rupture is clearly due to an effect on cell membrane, because it cannot be avoided by compounds that ordinarily stabilize against osmotic shock that would occur during lysozyme treatment.

3. Motility of B. subtilis during growth and sporulation. Both the standard strain and sporulation mutants of B. subtilis lose their motility at about t_3 or t_4 during the developmental period. Nevertheless, the cells can maintain a constant concentration of ATP. Only citric acid cycle mutants lose their ATP earlier, but at least for an aconitase mutant the concentration of ATP can be restored by glutamate and yet motility decreases at about t_4. Other experiments have shown that motility is coupled to the proton gradient of the cell because the uncoupler CCCP immediately destroys motility. The results indicate that an uncoupling process occurs during the developmental period but that it is not dependent on the early stages of development. Conversely, however, if this uncoupling does not occur, i.e., if motility does not decline, no sporulation has ever been observed. Thus, the uncoupling may be necessary although not sufficient for sporulation.

Significance to Biomedical Research and the Program of the Institute. The phenomenon of commitment is a general phenomenon observed in all organisms, and the finding that it can develop sequentially until an ultimate membrane stage is reached suggests that a similar sequential development of commitment may occur in higher organisms. More importantly, the development of commitment shows that membrane changes are extremely important for differentiation. Although the importance of phosphate for the glycerol metabolism is not completely understood, it is clear that the same phenomenon will apply to higher organisms which require exactly the same enzymes for phospholipid synthesis. The uncoupling of motility, and probably reactions such as amino acid transport from the proton gradient causing the destruction of the gradient itself during microbial differentiation can be related to the fact that thyroxine is also an uncoupler which initiates the differentiation from tadpoles to frogs. In both cases, the uncoupling process apparently is necessary but not sufficient for differentiation.

Proposed Course of Project: The mechanism by which phosphate interferes with the growth and lysis of glycerol mutant will be further investigated. The statistical analysis in the electron microscope has turned out to be more straight forward than we had anticipated so that we are now encouraged to investigate other developmental processes in a similar fashion. In particular, the correlation between the development of resistance to fusidic acid and the developmental membrane stage will be determined. Electron microscopy will also be used to investigate the molecular structure (microtubules) that assemble in HeLa cells in the presence of butyrate causing the appearance of long cellular processes which are usually observed in fibroblasts.

Keyword Descriptors:

Differentiation
Development
Sporulation
Electron Microscopy
Membrane Study
Glycerol-metabolism

Phospholipid-synthesis
Motility

Honors and Awards: None

Publications:

Freese, E.B., and Oh, Y.K.: ATP release and membrane collapses in glycerol-requiring mutants of Bacillus subtilis. J. Bacteriol., 120: 507-515, 1974.

Freese, E.B.: Studies of Suppression and Commitment to Sporulation. Symposium at the 1st Intersectional Congress of the JAMS, Toykyo, 1974.

Nishihara, T. and Freese, E.B.: Motility of Bacillus subtilis during growth and sporulation. J. Bacteriol., 1975 (in press)

Project No. Z01 NS 01962-03 LMB
1. Lab. of Molecular Biology
2. Section of Developmental
   Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mechanism and Control of Membrane Transport

Previous Serial Number: SAME

Principal Investigator: Ernst Freese, Ph.D.

Other Investigators: Martin Diesterhaft, Ph.D., Tadako Iijima, Ph.D., (Visiting Fellow), Barbara Levin, Ph.D., (Guest Worker)

Cooperating Units: None

Man Years

Total:	3.8
Professional:	3.3
Other:	0.5

Project Description

Objectives: Since we had previously characterized amino acid transport in cells and membrane vesicles of Bacillus subtilis and its inhibition by certain lipophilic acids, the following two projects appeared worthwhile: 1) Isolate mutants deficient in the transport of some amino acid, determine the specificity of this transport deficiency, and then attempt to use the difference between normal and mutant strains to isolate the carrier protein responsible for amino acid transport. The location of the mutations on the genetic map could also be determined and it could thus be decided whether one or more proteins were necessary for the transport of this particular amino acid. 2) Since hexachlorophene is known to cause brain deficiencies and to inhibit bacterial growth but the mechanism of these processes is not understood, use B. subtilis to determine the inhibitory effect of hexachlorophene and compare it with the effect of decanoate, a compound that inhibits growth by uncoupling amino acid from electron transport as we had previously shown.

Methods Employed: Mutants unable to transport glutamate or aspartate were isolated by their resistance to threo-hydroxy-aspartate. The binding of these amino acids to different membrane components was measured by equilibrium dialysis. An amino acid analyzer was used to determine the accumulation of intracellular amino acids. The inhibition of growth, oxygen consumption, ATP, or amino acid uptake was measured as usual. Genetic mapping was performed by PBSI transduction, with the threo-hydroxy-aspartate marker as donor.

Major Findings: 1. Characterization of the carrier in mutants deficient in aspartate/glutamate transport. Inhibition studies on the transport of aspartate and glutamate showed that the α- and γ- carboxy group and the amino group were required for the transport of these compounds. The following compounds did not inhibit aspartate or glutamate transport: N-methylaspartate, aspartate-α-methyl-ester or α-methyl-ester, the corresponding methylated glutamate compounds, bromosuccinate. Threo-hydroxy-aspartate was the only significant inhibitor of the transport of either amino acid. aspT mutants resistant to this compound had lost the ability to transport glutamate or aspartate while the transport of all other amino acids was completely unaffected. Genetic mapping of the aspT mutants showed that the corresponding mutation was always located in one particular genetic area, with the following tentative genetic map:

metC- metA- argC- aspT- glpD

It was attempted to label the carrier protein by the use of N-ethylmaleimide. To our surprise, the transport of glutamate was insensitive to treatment by this agent whereas the transport of malate was rapidly inactivated. Since the transport of aspartate was also inactivated, the aspartate/glutamate carrier apparently is located in the membrane in such a way that the aspartate attachment site is accessible to chemical treatment from the outside whereas the glutamate attachment site is not. While these results made it impossible to specifically label the carrier protein, they allowed an analysis of the carrier properties within the membrane.

2. Growth of B. subtilis on aspartate. In the course of these studies it was attempted to grow cells on aspartate as sole carbon source; but it was found that growth occurred at a reasonable rate only when the medium also contained sodium at an optimal concentration of 10 mM. This sodium effect was not related to the catabolism of aspartate because the biochemical reactions specific for this metabolism, i.e. aspartate transport, aspartase, aspartate transaminase, or fumarase, were not affected by sodium. However, in the presence of aspartate cells accumulated large amounts of homoserine which in turn was found to inhibit cell growth. While sodium addition could not reduce the amount of homoserine accumulated, it apparently could avoid the inhibition by this compound. A mutant able to grow much better than the normal strain on aspartate as sole carbon source was also isolated. Again sodium stimulated growth and homoserine was accumulated.

3. Mechanism of growth inhibition by hexachlorophene. Hexachlorophene is an extremely lipophilic compound which binds tenuously to the bacterial cells once it has been added to the medium. Thus, cells that are added subsequently or that have somehow escaped (by incomplete mixing) the attachment of hexachlorophene can grow while the other cells are inhibited. This situation is quite different from that previously observed for other lipophilic

acids, because these compounds, e.g., decanoate, partition between the bacteria and the medium so that a high fraction of the compound always remains in solution. Nevertheless, in most other respects hexachlorophene and decanoate had the same affect. They inhibited bacterial growth, oxygen consumption, ATP synthesis, and amino acid uptake in a medium containing citrate and amino acids as sole carbon source. However, when glucose was added to such cells, oxygen consumption, ATP synthesis, and growth eventually resumed. Nevertheless, amino acid uptake was still inhibited indicating that the amino acid transport was still uncoupled from the proton gradient and that ATP was regenerated by glycolysis. To our surprise, malate was similarly able to restore growth and very little inhibition was observed when the lipophilic acids were added to cells grown in malate as sole carbon source. Again, amino acid transport was not possible. Apparently, malate transport is not exclusively energized by the proton gradient of the cell and ATP can be generated via the succinyl-CoA synthetase although the ATP regeneration is still uncoupled from the electron transport system and so is as is the amino acid transport. The interesting conclusion is that antiseptics can be expected to be much effective in an environment that does not contain carbohydrates or other rapidly metabolizable carbon sources whose uptake is independent of the energization by proton gradient.

Significance to Biomedical Research and the Program of the Institute. Since membrane transport is universal phenomenon of all living cells the basic mechanisms of transport are likely to be the same in all biological systems. Thus, the transport of substrates into nerve cells is probably controlled by similar ion gradients (in this case Na^+) and the inhibitory mechanisms are also similar. The mechanism by which antiseptics inhibit cell growth are important because they permit the selection of different antiseptics for different environmental conditions and the use of antiseptics that are harmless to humans.

Proposed Course of Project: This particular project will be terminated at the end of this Fiscal Year but part of it will be continued under a new project.

Honors and Awards:

E. Freese received an award of the Environmental Mutagen Society for his work on the molecular mechanisms of mutations.

Publications:

Prasad, C.: Initiation of Spore Germination in Bacillus subtilis: Relationship to Inhibition of L-Alanine Metabolism. J. Bacteriol. 119: 805-810, 1974.

Taber, H., and Freese, E: Sporulation properties of cytochrome a-deficient mutants of Bacillus subtilis. J. Bacteriol. 120. 1004-1011, 1974.

Project No. Z01 NS 01963-04 LMB
1. Lab. of Molecular Biology
2. Section on Developmental Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Genetic Regulation in Human Diploid Tissue Culture

Previous Serial Number: Same

Principal Investigators: Richard C. Henneberry, Ph.D.
and Ernst Freese, Ph.D.

Other Investigators: Chang-Chih Tai, Ph.D. (Visiting Associate)
and E. Esat Atikkan, Ph.D. (Visiting Fellow)

Cooperating Units: Developmental and Metabolic Neurology Branch, NINCDS

Man Years:

Total:	3.5
Professional:	3.0
Other:	0.5

Project Description:

Objectives: The effect of lipophilic acids on growth and physiology of mammalian cells in culture has been examined. These compounds are used as preservatives and antiseptics, and some of them represent teratogenic drugs. Our previous studies have shown that fatty acids, regardless of chain length, inhibit the growth of both bacteria and mammalian cells. The inhibitory effect in bacteria can be attributed to uncoupling of keto- and amino-acid transport from electron transport. However, in mammalian cells neither amino acid transport nor ATP production are reduced by exposure to these compounds. Elucidation of the inhibitory mechanism(s) in mammalian cells remains one of the objectives of this project. Our earlier studies also revealed a phenomenon elicited only and specifically by short-chain fatty acids such as butyrate and propionate: cell morphology is strikingly altered by exposure to inhibitory concentrations of these compounds. We have shown that the extension of such neurite-like processes by HeLa cells is preceeded by an increase in the membrane ganglioside G_{M3} and by the induction of the biosynthetic enzyme for G_{M3}. During the period of this report, the mechanism (s) underlying the changes in morphology and membrane composition have therefore been examined in detail in both HeLa and Chinese Hamster Ovary (CHO) cells in response to both butyrate and butyryl-cAMP. The inhibition of these shape changes was also studied.

Methods Employed: 1. Morphological studies: Changes in cell shape due to exposure to various compounds have been studied by still phase micrography and phase cinemicrography. Methods for quantitative analysis of cinemicrographic data on morphological alterations have been developed and applied to studies on cell cycle synchronization and process elongation. (Simmons and Breuer, submitted for publication).

2. Growth inhibition studies: The growth inhibitory properties of fatty acids were measured by removing cells grown on petri plates with trypsin and counting viable cells with a hemocytometer or total cells in a Particle Data Counter. The inhibition has been correlated with the chain-length and partition coefficient of the fatty acid. Graphic presentation of these data permits prediction of the inhibitory concentration of other lipophilic acids.

3. Cyclic nucleotide transport studies: The coverslip monolayer method which we previously described for amino acid transport studies has been modified to permit rapid and accurate measurements of transport of cyclic nucleotides and their derivatives in mammalian cells.

Major Findings: 1. Enzyme induction studies: Studies on the butyrate-mediated induction of the ganglioside biosynthetic enzyme sialyltransferase have been extended to include an analysis of the biochemical properties of the induced enzyme (Fishman, Simmons, Brady and Freese, 1974, Biochem. Biophys. Res. Comm. 59: 292) (Fishman, Brady and Henneberry, manuscript in preparation). Studies on the biochemical properties of the sialidases in HeLa cells have been completed. Exposure of HeLa cells to butyrate was found to have no effect on the ganglioside degradation enzymes. (Tallman, Fishman and Henneberry, manuscript in preparation). These aspects of the project are reported more fully in Project Report Z01 NS 02164-05 DMN from the Developmental and Metabolic Neurology Branch, NINCDS.

2. Effects of divalent cation ionophores: Since microtubule assembly is needed for the extension of processes in HeLa cells and in the elongation of Chinese Hamster Ovary cells, and since high calcium prevents the assembly of microtubules, the effects of divalent cation ionophores on the inducible shape changes in these cells were examined. The calcium ionophore A23187 was found to prevent the shape changes induced by butyrate in HeLa cells and by mono- or di-butyryl cyclic AMP in CHO cells in a calcium-dependent manner. A slightly higher concentration of ionophore was needed to prevent the butyrate-mediated induction of sialyltransferase in HeLa. Ionophore treated cells continue to synthesize protein and remain viable. Our results suggest a role for calcium in the regulation of the sialyltransferase, in the regulation of microtubule assembly, and thus, in the control of cell morphology.

3. Reversal of butyrate-induced shape changes in HeLa: When butyrate is removed (i.e., the medium replaced by fresh one), the cells revert to normal dividing cells within 24 hr. However, two unexpected results have

provided new clues to the mechanism of action of butyrate as well as additional analytical tools:

a. When butyrate treated cells are removed by trypsinization and replated without butyrate, they again produce within 4 hr the long cell extensions.

b. When butyrate treated cells are exposed to fresh medium without butyrate but with low concentrations of cycloheximide which inhibit overall protein synthesis only partially, the cells retain their extended cell shape and they resume it after trypsination and replating. Inhibitors of RNA synthesis do not have this effect. Apparently, some protein, whose mRNA is already present, has to be made before the microtubular assembly can be prevented. This may be one of the proteins specifically needed for duplication.

4. Lack of involvement of cyclic nucleotides in butyrate-induced morphological changes: A comparison has shown that HeLa cells respond to butyrate but not to cAMP derivatives whereas Chinese Hamster Ovary cells respond to cAMP derivatives but not to butyrate. Thus, cyclic nucleotides do not play a role in the induction of membrane glycosphingolipid changes or shape changes in HeLa (Fishman, Simmons, and Henneberry, manuscript in preparation).

Significance to Biomedical Research and the Program of the Institute: Three aspects of the work on this project are of particular significance: 1. The effects of lipophilic acids, such as food additives and antiseptics on human cells in culture and a correlation with teratogenic effects of some drugs are directly applicable to questions of general health and abnormal development.

2. The induction of changes in membrane components by treatment with simple fatty acids relates to the question of membrane changes caused by malignant transformation and the so-called "reverse transformation" agents. Understanding of this phenomenon may aid in the identification of agents useful in the treatment of neoplastic growth.

3. Studies on the general question of regulation of cellular events are of basic importance in the understanding of normal and anomalous human development. In this regard, studies on the role of calcium in the regulation of microtubule assembly and the extension of neurite-like processes, the relationship between calcium and cyclic nucleotides as "second-messengers", and the control of induction of biosynthetic enzymes are pertinent examples.

Proposed Course of Project: The project as reported under the current Project Title will be terminated at the end of fiscal year 75 for the purpose of incorporation in a new project of broader scope. The following aspects of the current project will be pursued under the new Project Title:

1. Continued investigation into the mechanism of growth inhibition by lipophilic acids.

2. Studies on the level of biosynthesis at which sialyltransferase induction occurs and at which calcium ionophores inhibit induction.

3. Exploration of the mechanism by which small fatty acids elicit neurite-like process formation in HeLa cells and the relationship between this phenomenon and those produced by cyclic nucleotides in other cells.

4. Further studies on the control of microtubule assembly including the roles of calcium, cyclic nucleotides, and protein kinases.

Keyword Descriptors:

Morphology
HeLa cells
Chinese Hamster Ovary cells (CHO)
Butyrate
cyclicAMP
Gangliosides
Sialyltransferase
calcium ionophores

Honors and Awards: None

Publications:

Henneberry, R.C., Fishman, P.N., and Freese, E.: Morphological changes in cultured mammalian cells: prevention by the calcium ionophore A23187. Cell 5: 1-9, 1975.

Sheu, C.W., Salomon, D., Simmons, J.L., Sreevalsan, T., and Freese, E.: Inhibitory effects of lipophilic acids and related compounds on bacteria and mammalian cells. Antimicrobial Agents and Chemotherapy, 7: 349-363, 1975.

Simmons, J.L., Fishman, P.H., Freese, E. and Brady, R.O.: Morphological alterations and ganglioside sialyltransferase activity induced by small fatty acids in HeLa cells. J. Cell Biol., in press.

Project No. Z01 NS 02026-03 LMB
1. Lab. of Molecular Biology
2. Section on Molecular Virology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Regulation of Viral Nucleic Acids Synthesis in Animal Cells

Previous Serial Number: SAME

Principal Investigator: Robert A. Lazzarini, Ph.D.

Other Investigators: Leslye Johnson, M.S. (Chemist), Sabih Khan, Ph.D. (Guest Worker), Jack D. Keene, Ph.D. (Staff Fellow), Gudrun Stamminger, Ph.D. (Guest Worker), Michael Murphy, Ph.D. (Staff Fellow), Toshiro Adachi, Ph.D. (Visiting Fellow)

Cooperating Units: Laboratory of Biochemistry, NCI
Serial No. NCI/LB 379
Viral Oncology Branch, NCI

Man Years

Total: 5.5
Professional: 4.5
Other: 1.0

Project Description:

Objectives: The myxo-, paramyxo, and rhabdoviruses form a family of closely-related enveloped animal viruses which contain single-stranded RNA genomes. Although this group is responsible for many serious diseases, for example, influenza, rabies, mumps, and measles, very little is known about their replication. From the little that is known, it is clear that their mode of replication is different from that described for poliovirus, the RNA tumor viruses, or the RNA bacteriophages QB and MS2. The replication of the myxo-, paramyxo, rhabdoviruses is independent of host cell DNA function and takes place in the presence of actinomycin D. The single-stranded RNA viral genome is transcribed into a complementary RNA which serves as messenger RNA for the viral protein synthesis. This synthesis of complementary RNA is performed by an RNA-dependent RNA polymerase that is found in the virion itself. Later, in the infectious process, RNA of the same complementarity as the virion RNA is produced and packaged in the progeny virus particles. The details of the mechanism underlying the synthesis of RNA of both complementations senses and the regulation and coordination of these syntheses with each other are the subject of this project. Since the uninfected host cell does not carry out an RNA-dependent RNA synthesis, these viral processes probably represent the most accessible and specific target for therapeutic agents.

Major Findings: 1. Autointerference: The major thrust of our work during the last year concerned the phenomenon of autointerference. This aspect of the program was initiated during the previous year and some results reported in last year's Annual Report also are summarized briefly for continuity.

a. Historical Background: VSV and many other RNA viruses elaborate defective viral particles when infections are carried out at high multiplicities. These defective particles contain an RNA molecule that is smaller than that found in the infectious particle and are incapable of replication by themselves. They can replicate, however, when they co-infect a host cell with a standard infectious virus. During this joint replication, the defective particles are preferentially replicated and the progeny is enriched for the defective virus. There are two common types of defective VSV particles, the "T" particle which contains about 25% the normal complement of RNA and "LT" particle which contains 40% of the normal complement of RNA. However, as will be discussed below, the number of possible kinds of defective VSV particles is very large.

b. Characterization of RNA isolated from the "T" and "LT" particles: We have analyzed the RNA isolated from the two common classes of defective particles by RNA duplex reactions with viral mRNA isolated from infected cells. The results of these studies allow four important conclusions. 1) RNA of both defective interfering particles is viral and not host in origin. 2) The RNA from the defective particles is not a random assortment of viral RNA sequences of the appropriate length, but rather, each is a homogeneous population of molecules containing only part of the genetic information present in the whole VSV genome. 3) The two classes of defective RNA's show no sequence homology i.e., each RNA represents a distinct region of the genome with little or no overlap. This last result suggests that there is no specific genetic information needed for autointerference. 4) We are able to construct a rudimentary physical map of the VSV and DI particle genomes from our annealing data.

c. New class of defective interfering particles: The RNA in the defective particles described above is of the virion complementation sense and the particles can be considered deletion mutants of the standard virus. We have found another type of defective VSV particle that contains not only genomic RNA but also messenger RNA sequences. The genomic and messenger RNA sequences are complementary and covalently-linked to one another. RNA isolated from one type of these defective particles are partially double stranded. Because the two complementary sequences are covalently-linked, duplex formation between the sequences occurs in a concentration-independent fashion. RNA isolated from one of these defective particles is 80-90% double-stranded. Annealing and electronmicroscopic analyses confirm that the complementary sequences of this duplex are covalently-linked in a hairpin structure. Within the defective particle, however, the RNA exists as a single-stranded ribonucleoprotein complex 0.85μ in length. Upon proteinization the RNA collapses into the self-complementary double-stranded structure that is 0.33μ in length. Four other members of this new class of defective particles have been partially characterized. They differ principally in the amount of messenger RNA sequences contained. Significantly, they all contain part of the gene codes for the 28S RNA, suggesting that these defective particles may have resulted from the fragmentation of some specific intracellular viral structure rather than the random ligation of message and viral RNA's.

d. The mechanism of autointerference: The mechanism by which defective particles interfere with the production of the infectious virus has been the focus of much of the work carried out in this program. We have devised ways to measure the number of defective particles among the progeny of an autointerfered infection and are now able to describe the process in quantitative terms. Our results show that when 3 infectious VSV and 1 defective VSV infect a single cell, the resulting progeny consist of 3000 infectious particles and 6000 defective particles. We have altered the amount of the input defective particles (thus varying the degree of autointerference) and have examined the composition of the progeny particles. Our results show that over a wide range of autointerference, the number of progeny defective particles does not change, while the number of infective particles diminishes sharply with increasing interference. We have integrated these results into the following working hypothesis. The genome of the defective particle competes with that from the infective particle during replication. Consequently the amount of full-length genome accumulated in a cell diminishes in proportion to the degree of autointerference. Since the standard length genome is the template for mRNA synthesis in the infected cell, mRNA and consequently viral proteins, are limiting at high levels of autointerference. The constant number of progeny defective particles is a result of two opposing phenomena. The first is the potential increase in defective particle synthesis because of the favorable competition between the defective and infectious genomes, and the second is a general repression of all progeny particle synthesis because of restricted viral mRNA synthesis.

2. Structure-function relationships of viral components: We have continued our collaboration with the Laboratory of Biochemistry, NCI in a study of the structure function relationships of the VSV components. We have found that although VSV is classified as a bullet-shaped rhabdovirus, that shape is a result of artifacts that occur during the processing of the virus for visualization in the electronmicroscope. When VSV buds from the membrane of an infected cell it is bacillus - rather than bullet-shaped. The budding begins with the hemisphere containing the rounded end of the nucleocapsid and finishes with the completion of the non-nucleocapsid containing terminal hemisphere. The procedures used for concentrating and staining the virus disrupt the fragile terminal hemisphere and cause a collapse of part of the membrane forming the bullet-shaped particle. This finding resolves a curious dichotomy that exists in the literature concerning the shape of plant and

animal rhabdoviruses. Plant rhabdoviruses are considered to be bacillus with two hemispherical ends, while animal rhabdoviruses, typified by VSV, have been considered to be bullet-shaped with one plainer and one hemispherical end. It now appears that the difference in morphology between animal and plant rhabo-viruses may result from a difference in stability between plant and animal rhabdovirus membrane rather than a difference in the basic morphology of the two viruses.

Proposed Course of Project: 1. Further characterize the genome of the new defective interfering particles. This will entail determining whether the message sequences are at the 3' or 5' terminus of the RNA.

2. We will undertake an investigation to find normal replicative intermediates in infected cells that have structures similar to those found in the new class DI particles.

3. Further investigate the mechanism of autointerference. Attempts will be made to demonstrate whether any RNA suitably encased in the VSV N protein can interfere with the replication of the infectious virus or whether the specificity for this interference lies in the RNA. A study will be undertaken to determine the nature of the competitive advantage that the defective genome has compared to the infective genome.

4. Extend the electron microscopic studies to include examination of cells infected with normal virus as well as cells infected with defective virus.

Keyword Descriptors:

Vesicular Stomatitis Virus
Defective virus
Autointerference
RNA metabolism

Honors and Awards:

Invited speaker at the Gordon Conference on Animal Cells and Viruses, June 1975.

Invited speaker and session chairman at the 9th Benzon Symposium, Copenhagen, Denmark, 1975.

Publications:

Murphy, M.F., and Lazzarini, R.A.: Synthesis of viral mRNA and polyadenylate by a ribonucleoprotein complex from extracts of VSV-infected cells. Cell 3: 77-84, 1974.

Stamminger, G. and Lazzarini, R.A.: Analysis of the RNA of defective VSV particles. Cell 3: 85-93, 1974.

Ehrilich, H., Gallant, G., and Lazzarini, R.A.: Synthesis and turnover of Ribosomal RNA in quanine starved cells of E. coli. J. Biol. Chem., in press, 1975.

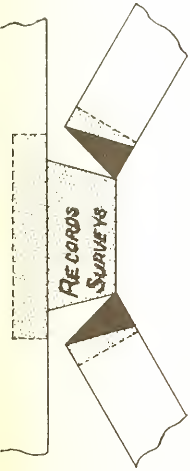
Lazzarini, R.A., Weber, G.H., Johnson, L.D., and Stamminger, G.M.: Covalently-linked message and anti-message (genomic) RNA from a defective vesicular stomatitis virus particle. J. Molecular Biol., in press, 1975.

OW TO USE
HESE SEPARATORS

se one page for
ach separation.

elect appropriate
ab, add further
dentification if
esired, and cover
t with scotch
ape.

ut off and discard
ll tabs except the
ne covered by tape.



TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

ANNUAL REPORT
July 1, 1974 through June 30, 1975
Laboratory of Neuro-otolaryngology, IR
National Institute of Neurological and
Communicative Disorders and Stroke

Jorgen Fex, M.D., Ph.D., Chief

SPACE

Out of the ten modules of the Laboratory, the modules 5D24, 5D26, 5D28, 5D30, and 5D32 were remodeled during the fall of 1974. Remodeling of module 5D16 will probably begin before July 1, 1975; the temporary installation (April, 1975) of our electron microscope will then become a permanent one and sinks will be added to the module for photography. Remodeling of the modules 5D18 and 5D20, also for morphology, and of 5D17 and 5D20 for biochemistry, is planned to start in the middle of August of 1975.

PERSONNEL

During Fiscal Year 1975 the following positions have been filled: two staff fellows, two senior staff fellows, biologist, electronic engineer, and clerk-typist.

During the spring of 1975, the following positions are being requested: biological laboratory technician (histopathology), computer specialist, chemist (biochemistry), and research chemist (biochemistry).

Due to the present personnel freeze, the requested positions will probably not be filled before July 1, 1975.

EQUIPMENT

The Laboratory has bought almost all costly equipment it now needs. This includes a computer, necessary for the physiological part of the program. Our choice in coping with the shortage of money at the beginning of the fiscal year was to order this computer half a year later than we first had planned; the physiology part of the program has therefore been delayed.

PROGRAM

The scientific activities of the Laboratory are promising.

The morphology section has worked out techniques for electron microscopic studies of the inner ear (chinchilla). It now produces pictures of cell junctions, including synapses, in the hearing organ. The resolution of details is remarkable; no other laboratory has produced better pictures. This advanced technique has led to new findings, which now are being evaluated in the first draft of a manuscript.

The biochemistry section has worked out micro-techniques for determining the activities of the transmitter-synthesizing enzymes, choline acetyltransferase, glutamate decarboxylase, and tyrosine hydroxylase in different parts of the auditory system (guinea pig). These are the initial steps in a neurochemical study of synaptic transmission in the mammalian inner ear and cochlear nucleus. The results will be evaluated in a manuscript at the beginning of the next fiscal year.

Experiments are also in progress in the biochemistry section regarding the classes of proteins of the stria vascularis and their enzymatic activities relevant to cochlear ion transport.

A study is in progress of the efferent pathways within the auditory system, including the termination of efferent nerve fibers in the cochlea and in the cochlear nucleus, using histochemical and autoradiographic techniques. When these efferent pathways have been traced, their physiology will be studied.

NON-PROJECT ACTIVITY

Together with the Director, Eaton-Peabody Laboratory of Auditory Physiology, Massachusetts Eye and Ear Infirmary, this Laboratory organized the international "Multidisciplinary Workshop on the Inner Ear and Hearing," October 8-11, 1974, sponsored by the National Institute of Neurological and Communicative Disorders and Stroke and the Fogarty International Center, held in Building 16 (Stone House), National Institutes of Health. Fifteen invited scientists participated with formal presentations; also, all investigators of this Laboratory were present at the meeting, listening and participating in discussions.

The purpose of the Workshop was twofold. First, specialists from different disciplines, all sharing an interest in the inner ear, were brought together so that all would bring back from the meeting a deeper and broader view of the mechanisms of the inner ear. Second, all the investigators of this Laboratory would get an excellent review of the state of art of all important aspects of inner ear research and also get acquainted with the best scientists in the field.

The Workshop was a success. No permanent record was taken of the proceedings and the participants therefore freely discussed unpublished material, both results and ideas. We hope that a similar meeting can be held in Fiscal Year 1977.

Project No. Z01 NS 02146 01 LNO
1. Laboratory of Neuro-otolaryngology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A biochemical study of synaptic transmission in the inner ear and cochlear nucleus

Previous Serial Number: None

Principal Investigators: Robert J. Wenthold, Ph.D.
Jorgen Fex, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total:	2.2
Professional:	1.8
Other:	.4

Project Description:

Objectives: To biochemically characterize synaptic transmission in the mammalian inner ear and cochlear nucleus.

Methods Employed: Initially, the activities of the transmitter-synthesizing enzymes, choline acetyltransferase, glutamate decarboxylase, and tyrosine hydroxylase are determined in the organ of Corti, spiral ganglion, auditory nerve, cochlear nucleus and inferior colliculus. Sensitive micro assays capable of detecting enzymatic activity in a few micrograms of tissue were developed.

Major Findings: Preliminary results indicate that choline acetyltransferase is moderately high in the cochlear nucleus and inferior colliculus, but low in the auditory nerve. Glutamate decarboxylase is high in the cochlear nucleus and inferior colliculus and low in the auditory nerve. Tyrosine hydroxylase is low in the cochlear nucleus, inferior colliculus and auditory nerve.

Significance to Biomedical Research and the Program of the Institute: Presently, there is little information concerning the nature of the afferent transmitter in the cochlea or the transmitter between the eighth nerve and the cochlear nucleus. Identification of these transmitters will greatly add to the understanding of auditory function and lend information to the field of synaptic transmission in general.

Proposed Course: The survey of choline acetyltransferase, glutamate decarboxylase, and tyrosine hydroxylase in the auditory system will be completed. Based on the outcome of these studies, additional experiments will be done to determine neurotransmitters active in the cochlea and cochlear nucleus. The identification of these transmitters will lead to experiments designed to elicit specific properties of synaptic transmission in the auditory system under normal and abnormal conditions.

Keyword Descriptors: Choline Acetyltransferase
Glutamate Decarboxylase
Tyrosine Hydroxylase
Neurotransmitters
Auditory System

Honors and Awards: None

Publications: None

Project No. Z01 NS 02147 01 LNO
1. Laboratory of Neuro-otolaryngology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Central connections of the auditory efferent system

Previous Serial Number: None

Principal Investigator: Joe C. Adams, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description:

Objectives: To determine the inputs to cells comprising the recurrent auditory pathways.

Methods Employed: Neural connections in cats and guinea pigs are studied using retrograde cell marking with horseradish peroxidase. The course and terminations of pathways are determined using autoradiographic procedures.

Major Findings: The project is in its beginning stages and there are not yet any major findings.

Significance to Biomedical Research and the Program of the Institute: The role of the efferent fibers of the auditory system is virtually unknown. Progress in understanding the role of these fibers should come with descriptions of their connections to other neural structures.

Proposed Course: When connections of the efferent system have been determined, electrophysiological studies will be initiated to study possible functional roles of the connections.

Keyword Descriptors: Auditory Efferent System
Horseradish Peroxidase
Autoradiography

Honors and Awards: None

Publications: None

Project No. Z01 NS 02148 01 LNO
1. Laboratory of Neuro-otolaryngology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Isolation and characterization of cochlear transport proteins

Previous Serial Number: None

Principal Investigator: Dennis G. Drescher, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.1
Professional:	.7
Other:	.4

Project Description:

Objectives: To characterize the properties of proteins responsible for ion compartmentalization and resting potentials within the inner ear, and relate these properties to cochlear function and dysfunction.

Methods Employed: Initially, non-cochlear adenosine triphosphatase and carbonic anhydrase of guinea pig will be purified by usual preparatory techniques including treatment with salts and detergents, column chromatography, and preparative electrophoresis. The isolation procedures will then be scaled down to accommodate pooled samples of stria vascularis. The properties of the strial and non-strial enzymes will be compared by various methods including analytical disc gel electrophoresis.

The properties of the strial ATPase and strial carbonic anhydrase obtained from guinea pigs will be correlated with the physiological state of the cochlea by measurement of endocochlear potential and endolymphatic concentrations of sodium and potassium.

Major Findings: As a new project in a newly established laboratory, initial effort has been expended mainly in ordering and setting up necessary equipment, and no major experimental results have been obtained.

Significance to Biomedical Research and the Program of the Institute: The mechanism as to how the inner ear transforms acoustic signals into electrical signals is as yet poorly understood. An understanding of cochlear active transport systems at the molecular level should shed light on how such transformation is accomplished in the normal cochlea and how it can be perturbed in cochlear abnormalities.

Proposed Course: Initial experiments will involve working out methods of protein isolation and electrophysiological measurement outlined above. In general, continued attention will be given to establishing a functioning laboratory.

Keyword Descriptors:	Adenosine Triphosphatase Electrophoresis Stria Vascularis Carbonic Anhydrase Endocochlear Potential Cochlear Active Transport

Honors and Awards:

Invited speaker at the International Symposium on the Effects of Noise on Hearing--Critical Issues, June 23-25, 1975, at Cazenovia, New York, giving talk entitled "Review of General Cochlear Biochemistry in Normal and Noise-Exposed Ears," to be published by Raven Press.

Publications: None

Project No. Z01 NS 02149 01 LNO
1. Laboratory of Neuro-otolaryngology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A morphological study of the junctions of the inner ear, including the synapses

Previous Serial Number: None

Principal Investigator: Robert L. Gulley, Ph.D.

Other Investigators: Thomas S. Reese, M.D.

Cooperating Units: Laboratory of Neuropathology and Neuroanatomical Sciences, IR, NINCDS

Man Years:

Total:	2.0
Professional:	.9
Other:	1.1

Project Description:

Objectives: To study morphologically the interrelationship of the cellular components of the inner ear and to determine the role of the various interactions in the function of the organ of Corti.

Methods Employed: The cochleas of chinchillas were used for the study. The cochleas were fixed by simultaneous perfusion of the cochleas and vascular systems. The fixatives were either a combination of aldehydes followed by postfixation in osmium tetroxide or a Millonig's fixative. The tissue was stained "en bloc" in uranyl acetate. After preparing one-micron sections to verify the plane of section, thin sections were examined in the electron microscope using either a biological or goniometer stage. Some cochleas were processed for the freeze-fracture technique on a Balzers 360 freeze-fracture unit.

Major Findings: Both freeze fracture and thin sectioned material were examined for the following regions of the inner ear: 1) reticular lamina, 2) synaptic region of the inner and outer hair cells, 3) stria vascularis, and 4) supporting cell region of the organ of Corti.

The reticular lamina has two major types of cell contacts: 1) hair cell-supporting cell and 2) supporting cell-supporting cell junctions. Each cell type appears to have a characteristic distribution of fibrillar material associated with the junctional complex. All of the contacts have regions of tight junctions. In thin section the hair cell-supporting cell junction has a region of tight junction at the endolymphatic luminal surface. Below this the membranes are separated by 250-300 Å. Throughout the junction small puncta of tight membrane apposition occur. In freeze-fracture preparations the luminal membrane has from four to six rows of ridges on the A face parallel to the lumen. Below this three or four parallel ridges are arranged in a honeycomb mosaic pattern extending to the abluminal membrane. The supporting cell-supporting cell junctions appear in three forms: The Deiter-Deiter cell junction has a tight junction at the luminal surface. The next one to two microns has the appearance of an intermediate junction and tufts of fibrillar material closely associated with the inner leaflet with a regular periodicity. Occasional areas of tight membrane apposition are found in the upper portion of the junction. In freeze fracture a junction similar to the hair cell-supporting cell junction is found near the luminal surface with one major difference. The pattern of ridges in the honeycomb mosaic is less dense and the strands are composed of single ridges. Parallel ridges of large particles have been seen on the abluminal A face membranes. The periodicity of the ridges compares well with that of the clumps of fibrillar material seen in that region of the junction. The region of membrane apposition between the inner phalangeal cells is narrow ($\sim 0.2\ \mu$) and is a tight junction. In freeze fracture the junction consists only of four or five parallel ridges with no honeycomb mosaic. The pillar-pillar cell junction is unique. While the luminal zone is similar to that in the inner phalangeal cells, the abluminal membranes have randomly spaced gap junctions. In freeze fracture replicas these take two forms, one a small ($\sim 0.08\ \mu$) cluster of particles on the A face arranged asymmetrically around an evagination of the surface and the second a macular tight junction. Both of these have also been seen in thin section.

Most of the major types of synaptic interactions have been described in both freeze fracture and thin sectioned material. In thin sections there appeared to be significant differences between the inner hair cell-afferent and outer hair cell-afferent synapse. There does not appear to be an accumulation of synaptic vesicles near the presynaptic junctional area in the OHC-afferent synapse; however, freeze fracture replicas suggest the synapse is chemical. The efferent-hair cell and efferent-afferent synapse morphologically resemble what has been characterized as a cholinergic excitatory ending. The afferent A face does appear to have some specialization in regions of synaptic interaction.

The stria vascularis has been examined with freeze fracture and to a limited extent in thin sections. While a correlation of these two images remains to be completed, this area appears rich in interesting junctional and membrane specializations.

While there is no evidence of gap junctions between hair cells or hair cells and supporting cells, there are numerous examples of this junction between supporting cells. Most commonly, the junctions occur between Deiter cells directly underneath the outer hair cells and surrounding the afferent nerve fibers. This structure has been confirmed in both freeze fracture and thin sectioned material

Significance to Biomedical Research and the Program of the Institute: These studies represent the first systematic characterization of junctions between the various cellular elements of the organ of Corti. Information of the interaction of the various elements undoubtedly will shed new light on transducer function of the organ of Corti and the role of the stria vascularis. A ground has been laid for further morphological, biochemical and functional studies of the basis for function and dysfunction in the adult and developing auditory system.

Proposed Course: Completion of the study on the junctions present in the inner ear with emphasis on the synaptic junctions is the first priority. Experiments are anticipated to delineate the functional significance of these structures. The information will serve as a basis for describing normal and abnormal development of the inner ear.

Keyword Descriptors: Organ of Corti
Cellular Junctions
Synapses

Honors and Awards: None

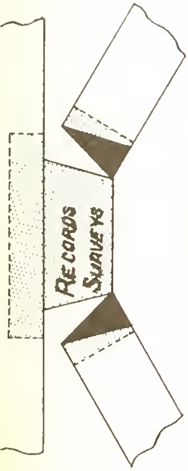
Publications: None

OW TO USE
HESE SEPARATORS

se one page for
ach separation.

elect appropriate
ab, add further
dentification if
esired, and cover
t with scotch
ape.

ut off and discard
ll tabs except the
ne covered by tape.



TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

Annual Report
July 1, 1974 through June 30, 1975
Laboratory of Neuropharmacology

The Laboratory of Neuropharmacology was established during the past year to conduct basic and clinically applied studies of central synaptic mechanisms and their relation to the pathogenesis of disorders of motor, sensory and cognitive function. The major goal of the laboratory is to develop improved pharmacologic therapies for neurologic disease. Experimental efforts within the Laboratory will focus on synaptic mechanisms since substances participating in transmission act as sensitive indicators of central nervous system function and constitute the site of action of many drugs which affect psychomotor behavior. Alterations in monoamines and other central neurotransmitters have been found in relation to several extrapyramidal disorders and have lead to the discovery of drugs which reverse both the biochemical and clinical defects. This approach may lead to the development of new treatments for other disorders of brain function whose symptoms reflect an aberration in central synaptic processes.

The Laboratory has tentatively been organized into four units: Experimental Therapeutics, Neurophysiology, and Anatomy and Biochemistry for which staff are now being recruited. This vertically integrated structure should facilitate the rapid translation of basic information into improved therapeutic modalities and promote the expeditious preclinical testing of hypotheses deriving from clinical studies. Implementation of the Laboratory's research programs remain incomplete since only about one-half of the Laboratory's personnel and space allocations have yet been filled.

Initial research activities have focused on central neural systems implicated in the pathogenesis of extrapyramidal disorders, especially Parkinson's disease, Huntington's chorea and tardive dyskinesia. Considerable indirect evidence links the development of these disorders with dysfunction of neural pathways utilizing monoamines, acetylcholine or GABA. With the ultimate aim of pharmacologically manipulating these systems and thus compensating for the defect in synaptic transmission, the Laboratory has been investigating mechanisms which regulate the synthesis and post synaptic efficacy of catecholamines and GABA as well as the functional interrelationships of neuronal networks containing these transmitters.

I. Neuropharmacology of the GABA system

A. Clinical Studies

Recent reports of diminished levels of GABA and of its synthesizing enzyme, glutamic acid decarboxylase, in the brains of patients with Huntington's disease have prompted various pharmacologic attempts to augment GABA mediated synaptic transmission within the human central nervous system. In view of the possibility that the barrier to GABA transport into the CNS in part derives from the degradation of the amino acid by transamination, clinical trials of high doses of orally administered GABA in combination with dipropylacetate, a potent GABA transaminase inhibitor, were conducted in patients with Huntington's chorea. No centrally mediated toxicity was observed, nor, unfortunately was there any therapeutic effect. In another clinical trial, the putative GABA analogue, imidazole-4-acetic acid also failed to produce alterations in motor or cognitive function in a group of Huntington's chorea patients. It remains to be determined, however, whether this drug actually entered the human central nervous system. HA-966, a structural analogue of GABA which in animals increases striatal dopamine levels, also produced no significant clinical change in patients with either Parkinson's disease or Huntington's chorea. Since it has not been established that HA-966 is a GABA receptor stimulator, this negative result, as well as those obtained with GABA plus dipropyl acetate, or with imidazole acetic acid cannot be construed as casting doubt on the hypothesis that a defect in GABA mediated transmission plays a pathogenetic role in Huntington's disease. The central turnover of both dopamine and serotonin appeared to be increased during the systemic administration of GABA plus dipropyl acetate to Huntington's chorea patients, suggesting that a highly interacting relationship exists between GABA, serotonin and dopamine-containing neural pathways in the human central nervous system; pharmacologic exploitation of these interactions are now planned.

B. Preclinical Studies

Preclinical studies have centered on factors controlling the activity of glutamic acid decarboxylase, the rate limiting enzyme in GABA synthesis. The enzyme's substrate, glutamate, has been found to interfere with binding of the cofactor pyridoxal phosphate in a dialyzed brain homogenate preparation, suggesting that the balance between substrate and cofactor concentrations at some critical intraneuronal site might be significant in GABA synthesis in vivo. Moreover, kinetic differences appear to exist between the soluble and bound enzyme. The significance of these different states and their possible regulation by changes in neuronal activity is currently being explored.

In rat pineal, glutamic acid decarboxylase activity has not been found to be altered by manipulations which affect other pineal functions, such as changes in norepinephrine levels and the diurnal cycle, superior cervical gangliectomy or habenulectomy. These results suggest that the enzyme may be confined to glial cells within the pineal. Glia in other parts of the central nervous system have been shown to actively accumulate and metabolize GABA and an important question emerging from these considerations is whether the glial pool of GABA can be influenced to yield increased extracellular concentrations of the amino acid at sites of neuronal degeneration.

Considerable evidence supports the hypothesis that the activity of dopamine neurons whose cell bodies are located in the pars compacta of substantia nigra is regulated by a striato-nigral feedback loop which utilizes GABA as a transmitter. To explore the possible pharmacological significance of this interaction, a variety of drugs known to influence the functioning of dopaminergic systems are being tested for their ability to alter GABA turnover. To date these drugs have not appeared to affect GABA metabolism. However, it appears that the use of more sensitive and short-term indices of GABA turnover can resolve effects which the long-term studies have not demonstrated and this possibility is now being pursued.

II. Neuropharmacology of the Dopamine System

A. Clinical Studies

Investigations of dopaminergic mechanisms and the central nervous system of man have involved three different approaches. In one, an improved understanding of the "on-off" phenomena which complicates chronic treatment of parkinsonism with L-dopa has been sought. These studies suggest that central noradrenergic mechanisms as well as alterations in circulating dopa levels may contribute to this disabling response to L-dopa.

The search for better drug therapy for Parkinson's disease constitutes a second approach. This has lead to a clinical trial of bromocriptine, an ergot derivitive known to directly activate post-synaptic dopamine receptors. Initial results indicate that bromocriptine may be at least equivalent to L-dopa in antiparkinsonian efficacy. In patients receiving therapeutically optimal dose levels of L-dopa or peribedil, neither clonidine (an α-adrenergic agonist) nor caffeine (a phosphodiesterase inibitor), either of which substantially increases the effects of dopaminergic agents on motor function in the rat, exerted any significant effect on extrapyramidal function. These results cast doubt on the value of the L-dopa potentiation test in rodents as a predictor of therapeutic efficacy in parkinsonian patients.

The third approach to investigating dopaminergic mechanisms in man has involved studying the influence of this system on the release of pituitary hormones. The results suggest that supersensitivity of dopamine receptors in the tuberohypophyseal pathway may occur in patients with Huntington's chorea or tartive dyskinesia. Studies involving the use of fenfluramine, a serotonin agonist, have implicated the serotonin system in the regulation of release mechanisms for prolactin and to a lesser extent for growth hormone in man.

B. Preclinical Studies

The past year has witnessed the initiation of studies of the responses of the rodent dopamine system to pharmacological and mechanical alterations of specific aspects of synaptic function. By combining both biochemical and single unit recording techniques it is becoming possible to gain a better understanding of the range of neuronal compensatory responses. An evaluation of changes in dopamine synthesis in various parts of the dopamine neuron after inhibition of impulse flow has shown that cell bodies and terminal areas respond differently. The increases in dopamine synthesis which occurs in terminal regions after cessation of neuronal activity can be blocked by drugs which stimulate dopamine receptors. This observation combined with microdissection techniques and a sensitive dopamine assay makes it possible now to look for regional differences in mechanisms regulating dopamine synthesis as well as in the drug sensitivities of presynaptic dopamine receptors in the various areas receiving dopamine input.

Dopamine containing neurons in the substantia nigra and ventral tegmental areas of the rat are spontaneously active in anesthetized preparations. Drugs which activate post-synaptic dopamine receptors have been found to diminish the activity of these cells possibly reflecting post-synaptic influences mediated by the striato-nigral feedback loop. This system provides a sensitive model for evaluating drugs which may interact with dopamine receptors and should facilitate preclinical screening of dopamine agonists. In the future such studies will be combined with the investigation of other parts of the extrapyramidal system with the hope of identifying additional sites where symptoms attending the failure of the dopamine system might be alleviated by drug intervention.

Project No. Z01 NS 02139-01 LP
1. Laboratory of Neuropharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biochemistry, Pharmacology and Physiology of Monoamines and Other Central Neurotransmitters

Previous Serial Number: None

Principal Investigator: Thomas N. Chase
Donald B. Calne

Other Investigators: Ronald Kartzinel
Marina M. Mata
Leonard P. Miller
Danka Pericic
Mark J. Perlow
Ira Shoulson
Judith R. Walters
Roger L. Weir (Guest Worker)

Cooperating Units: Laboratory of Clinical Science, NIMH
Behavioral Biology Branch, NINCHD
Laboratory of Clinical Investigation, NIAID
City of Hope National Medical Center, Duarte California
Department of Medicine, Downstate Medical College, Brooklyn, New York
Department of Psychiatry, Yale University Medical Center, New Haven, Connecticut
Division of Pediatrics and Neurology, The Mirian Hospital, Providence, Rhode Island

Man Years:

Total: 8
Professional: 6
Others: 2

Project Description:

Objectives: (1) To study the transport, metabolism, storage, release and post-synaptic interactions of monoamines and other central neurotransmitters. (2) To examine the functional role of monoamines and related neurohumoral agents in the central nervous system of normal individuals and in patients manifesting neurologic disease. (3) To study the mechanism of action and therapeutic efficacy and toxicity of drugs which modify brain function.

Methods Employed: (1) Preclinical studies utilize (a) single unit recording techniques for determining the effects of drugs on the activity of identified neurons in the brain of laboratory animals; (b) homogenates of brain, peripheral nerve, and other tissues from control or drug treated animals which are analyzed for enzyme activity and levels of monoamines, cyclic neucliotides or other substances participating in synaptic transmission; (c) surgically prepared laboratory animals in which the concentration of labeled and endogenous compounds in brain, cerebrospinal fluid, serum, and urine are determined under various experimental conditions following the administration of labeled amines and other substances. (2) Clinical studies are conducted in patients with neurologic disorders and in normal volunteers. Under basal conditions and during the administration of drugs or various physical procedures believed to influence central synaptic mechanisms, neurologic, psychiatric and psychological parameters of brain function are correlated with results of (a) chemical assay or relevant compounds in various body fluids and tissues, (b) radioisotopic tracer procedures and (c) electrical measures of spontaneous and evoked cortical potentials.

Major Findings: Preclinical investigations conducted with Dr. Miller have attempted to resolve some newly observed properties of glutamic acid decarboxylase (GAD), the rate limiting enzyme in GABA synthesis. Studies of a dialyzed preparation of GAD suggest that residual enzyme activity is due to non-dialyzable pyridoxal phosphate which can be removed by incubation in the presence of the enzyme's substrate, glutamate. In addition, evidence suggests the enzyme may have two kinetic states. After homogenization and dialysis in the presence of a solubilizing agent (Triton X-100), an altered Km for glutamate was observed implying that there may be important kinetic differences between the soluble and bound enzyme.

Dr. Mata, in collaboration with Drs. Schrer and Kline, of the NICHD Behavioral Biology Branch, has been studying the properties of GAD in rat pineal and choroid plexus. GAD activity in the pineal does not appear to be affected by changes in norepinephrine concentrations, the diurnal cycle, superior cervical gangliectomy or habenulectomy. The enzyme remains reasonably stable in organ culture and may reside within glial cells. High levels of GAD activity have also been found in rat choroid plexus.

Dr. Perlow, in collaboration with Drs. K. Black, NIMH Laboratory of Clinical Science, and S. Wolff, NIAID Laboratory of Clinical Investigation, has examined neurochemical changes in rabbit hypothalamus, platelets and plasma associated with endotoxin-induced fever. Available results suggest that during defervescence there is a reduction in hypothalamic serotonin, norepinephrine and dopamine as well as a decline in platelet serotonin. In unanesthetized monkeys, Dr. Perlow has been able to elicit a febrile response with leucocytic pyrogen obtained from human white blood cells. This response varies with the time of day in which the fever is initiated and is suppressed by prostaglandin synthesis inhibitors.

Dr. Walters has begun studies of the effects of drugs on single-unit activity of dopamine neurons and of neurons in CNS areas influenced by the dopamine system. Preliminary results indicated that drugs (such as apomorphine or piribedil) which act directly to stimulate dopamine receptors, diminish the firing of dopamine cell bodies located in the substantia nigra. This recording technique will be further applied to the evaluation of potential dopamine agonists and the determination of their effects on the firing rates of individual cells in areas postsynaptic to dopamine neurons such as the globus pallidus.

Dr. Pericic has combined a newly devised microdissection technique with a sensitive dopamine assay to study the effects of drugs and neuronal activity on dopamine levels in the limbic cortex and subregions of the substantia nigra. It would now appear that inhibition of impulse flow in dopamine neurons in these areas does not increase dopamine levels in either the pars compacta or pars reticulata of substantia nigra as it does in the striatum and limbic cortex. These observations suggest that if, as recently suggested, dopamine dendrites make synapses onto other neuronal elements, the properties of these synapses are different from those made by dopamine axons. The ability of various drugs to affect the increase in dopamine observed in the cortex and caudate after inhibition of impulse flow in dopamine neurons will now be studied to determine whether differences exist in the way presynaptic dopamine receptors in these two areas respond to drugs.

To compliment studies on the central regulation of dopamine and GABA synthesis, interactions between dopamine and GABA containing neural systems are now under investigation by Drs. Walters, Mata and Pericic. Several drugs known to affect dopaminergic functioning have been tested for their ability to alter GABA turnover, as estimated by the accumulation of this amino acid following the administration of aminooxy acetic acid. Neither chronic nor acute L-dopa treatment nor the acute administration of haloperidol, α-methyltyrosine, apomorphine, reserpine or thioridazine produced any change in GABA metabolism. The development of a more sensitive and short-term index of GABA turnover utilizing labeled precursors and gas chromatography is now underway.

Seven parkinsonian patients who develop severe "on-off" effects during chronic treatment with L-dopa have now been studied with Drs. Shoulson and Glaubiger. Marked swings in plasma dopa levels as well as in motor function followed each oral dose of L-dopa. A constant intravenous infusion of L-dopa resulted in stable plasma dopa concentrations and virtual disappearance of motor fluctuations. Notwithstanding steady plasma dopa levels, an abrupt decline in the antiparkinsonian response to L-dopa attended postural tilting or cold pressor stimulation. Although these maneuvers were accompanied by a significant rise in plasma norepinephrine, the intravenous infusion of this catecholamine failed to influence the effect of L-dopa on parkinsonian signs. The results suggest that central noradrenergic mechanisms as well as alterations in circulating dopa levels may contribute to the on-off response to L-dopa.

Clinical trials of bromocriptine, an ergot derivitive which has been shown to activate postsynaptic dopamine receptors, has been initiated by Drs. Calne and Kartzinel. The enzyme responsible for converting L-dopa to dopamine is depleted in the brain of parkinsonian patients so that a therapeutic advantage may be predicted for bromocriptine which does not require enzymatic transformation for therapeutic activity. Initial results indicate that bromocriptine is at least equivalent in antiparkinsonian efficacy to L-dopa. In addition, this drug provides a useful tool for analysis of mechanisms responsible for such adverse reactions to L-dopa as the on-off phenomenon.

Studies of the effects of orally administered caffeine on the antiparkinsonian efficacy of L-dopa or piribedil was studied in six patients by Dr. Shoulson. At doses which induced unequivocal central nervous system stimulation, caffeine produced no change in the therapeutic response to either antiparkinsonian agent. On the other hand, patients with L-dopa dyskinesias reported an increase in the duration of their involuntary movements with caffeine co-administration. Since in the experimental animal caffeine is known to stimulate both dopaminergic mechanisms and motor activity, the present results cast doubt on the value of these factors as predictors of therapeutic efficacy in parkinsonian patients.

An evaluation of the ability of orally administered clonidine, an α-adrenergic agonist, to modify the therapeutic efficacy of L-dopa or piribedil has now been completed in parkinsonian patients together with Dr. Shoulson. At doses which produce unequivocal cardiovascular changes, clonidine significantly diminished the antiparkinsonian potency of either drug. Anticholinergic agents markedly reduced this effect of clonidine. The results suggest that clonidine possesses cholinergic as well as noradrenergic activity and cast further doubt on the accuracy of the L-dopa potentiation test in rodents as a predictor of therapeutic efficacy in patients with Parkinson's disease.

Dr. Shoulson, in collaboration with Dr. Carter of the Downstate Medical College, has continued clinical studies of the influence of monoaminergic systems on the release of pituitary hormones. Treatment with L-dopa in combination with a peripheral decarboxylase inhibitor significantly depressed plasma prolactin but elevated growth hormone levels in normal volunteers as well as in patients with various extrapyramidal disorders. Individuals with Huntington's disease or tardive dyskinesia showed a significantly greater response to treatment with the dopamine receptor agonist, piribedil, than did normal control subjects. These latter results suggest that supersensitivity of dopamine receptors may attend certain central nervous system disorders.

In order to evaluate the influence of the serotonin system in the regulation pituitary hormone release mechanisms, Dr. Shoulson has also examined changes in prolactin levels in man and in laboratory animals following the administration of fenfluramine, a drug known to facilitate serotonin mediated function. Fenfluramine produced a significant increase in prolactin levels in normal volunteers as well as modest rise growth hormone levels.

A double blind study of orally administered fenfluramine was completed with Dr. Shoulson in a group of non-obese adults with various neurologic disorders. Caloric intake and body weight fell significantly after eight days of treatment. Average central turnover of serotonin, as estimated by the CSF accumulation of 5HIAA during probenecid loading, decreased by 66%. No significant change in HVA, the major dopamine catabolite, occurred. The results support the contention that the effect of fenfluramine on human dietary intake may be mediated by alterations in serotonergic rather than dopaminergic mechanisms.

Lumbar spinal fluid levels of HVA and 5HIAA, the major metabolites of dopamine and serotonin respectively, in hyperactive children did not differ significantly from age matched controls. In these studies carried out in collaboration with Dr. Shetty at the Rhode Island Hospital, therapeutic dose levels of d-amphetamine reduced the spinal fluid content of HVA but not of 5HIAA. No change in either monoamine metabolite occurred with placebo therapy. In hyperactive children receiving d-amphetamine, the amount of HVA decline correlated well with the degree of clinical improvement. The results suggest that hypofunction of dopamine mediated synaptic function may occur in the CNS of children manifesting the hyperactive syndrome.

Recent reports of diminished GABA and glutamic acid decarboxylase levels in the brains of patients with Huntington's disease suggest that certain symptoms of this disorder may reflect degeneration of central GABA containing neurons. Therapeutic trials were therefore conducted with imidazole-4 acetic acid, a drug which is believed to act to directly stimulate post synaptic GABA receptors, in collaboration with Dr. Roberts. Unfortunately, this agent as well as its isopropylester derivitive failed to produce any alteration in motor or cognitive function in a group of Huntington's chorea patients. Whether the drug actually entered the central nervous system of man has not yet, however, been firmly established.

A study with HA966 (1-hydroxy-3-amino pyrolidine-2), a structural analog of GABA which increases dopamine levels in the striatum of animals, was conducted with Dr. Kartzinel in patients with various extrapyramidol disorders. No significant change in clinical state was observed in five individuals with Parkinson's disease or in five with Huntington's chorea.

The failure of systemically administered GABA to alleviate the symptoms of Huntington's disease has been attributed to the relative inability of this amino acid to penetrate the blood brain barrier. In view of the possibility that the barrier to GABA transport into the central nervous system was due in part to degradation by transamination, clinical trials of systemically administered GABA in combination with the GABA transaminase inhibitor, dipropylacetate, were conducted with Dr. Shoulson in patients with Huntington's disease. No therapeutic or centrally mediated toxic effects of this drug combination were observed. The central turnover of both dopamine and serotonin did, however, appear to be significantly increased during this treatment, perhaps indicating that a highly interactive relationship exists between GABA, serotonin and dopamine containing neuronal pathways in the human central nervous system.

Key Words

Neuropharmacology, Neurophysiology, Neurochemistry, Neuroendocrinology, Dopamine, Norepinephrine, Serotonin, Acetylcholine, γ aminobutyric acid, Parkinson's disease, Huntington's chorea, L-dopa, Cerebrospinal fluid,

Honors and Awards

Dr. Chase served as an invited participant and Session Chairman at the Karolinska Institute Symposium on Antipsychotic Drugs, Stockholm, September 17-19, and was appointed to the Board of Scientific Advisors of the Tourrette Foundation.

Dr. Calne has been appointed as a member of the Advisory Board of the American Health Foundation's Official Journal of Preventative Medicine and to the Advisory Board of the United Parkinson's Foundation.

Dr. Walters was awarded a Workshop Fellowship from the Huntington's Disease Foundation.

Publications

Chase, T.N., Woods, A.C. and Glaubiger, G.A.: Parkinson disease treated with a suspected dopamine receptor agonist. Arch. Neurol. 30:383-386, 1974.

Perez-Cruet, J., Chase, T.N. and Murphy, D.L.: Dietary regulation of brain tryptophan metabolism by plasma ratio of free tryptophan and neutral amino acids in humans. Nature 248:693-695, 1974.

Carman, J.S., Shoulson, I. and Chase, T.N.: Huntington's chorea treated with lithium carbonate. Lancet i: 811, 1974.

Chase, T.N.: Serotonergic-dopaminergic interactions and extrapyramidal function. In Costa, E., Gessa, G.L. and Sandler, M. (Eds.): Serotonin-New Vistas. Advances in Biochemical Psychopharmacology, Vol. 11, Raven Press, New York, pp. 377-385, 1974.

Chase, T.N., Woods, A.C., Lipton, M.A. and Morris, C.E.: Hypothalamic releasing factors and Parkinson disease. Arch. Neurol. 31:55-56, 1974.

Chase, T.N.: Fusaric Acid in Parkinson's disease. Neurology 24: 637-639, 1974.

Chase, T.N.: Clinical studies of dopaminergic mechanisms. In Usdin, E. (Ed.): Neuropsychopharmacology of Monoamines and Their Regulatory Enzymes, Raven Press, New York, pp. 427-434, 1974.

Eng, N. and Chase, T.N.: Measurement of homovanillic acid in cerebrospinal fluid. In Sunderman, F.W. (Ed.): Manual of Procedures for the Laboratory Diagnosis of Skeletal, Muscular and Nervous Disorders, pp. 143-148, 1974.

Shoulson, I. and Chase, T.N.: Fenfluramine and dyskinesias. New Eng. J. Med. 291:850-851, 1974.

Chase, T.N., Jacobowitz, D.M. and Balch, T.S.: Dopamine histofluorescence in post mortem specimens of human and rat striatum. J. Psychiat. Res. 11:281-282, 1974.

Chase, T.N.: Clinical studies of dopaminergic mechanisms. Psychopharmacol. Bull. 10:43-44, 1974.

Calne, D.B., Chase, T.N. and Barbeau, A. (Eds.): Advances in Neurology, Vol. 9, Dopaminergic Mechanisms, Raven Press, New York, 1975.

Chase, T.N. and Shoulson, I.: Dopaminergic mechanisms in patients with extrapyramidal disease. In Calne, D.B., Chase, T.N. and Barbeau, A. (Eds.): Advances in Neurology, Vol. 9, Raven Press, New York, pp. 359-366, 1975.

Shoulson, I. and Chase, T.N.: Huntington's disease. Ann. Rev. Med. 26:419-426, 1975.

Chase, T.N. and Shoulson, I.: Behavioral and motor effects of

ANNUAL REPORT
July 1, 1974 through June 30, 1975
Infectious Diseases Branch, IR
National Institute of Neurological and
Communicative Disorders and Stroke

I. Responsibility of the Branch

The responsibility of the Infectious Diseases Branch is to carry out planned directed research programs concerned with infections which damage the human nervous system. The Branch is divided into three sections: 1) Immunochemistry and Clinical Investigations, 2) Virology and Bacteriology, and 3) Experimental Pathology. These sections utilize the techniques of immunology, clinical investigations including human volunteers and clinical trials; virology, bacteriology, mycoplasmaology, neurovirology and electron microscopy; as well as experimental pathology with non-human primates.

II. Program Segments

The program segments are: a) Perinatal, b) Acute and c) Chronic. In each segment we are concerned with 1) etiology and diagnosis, 2) treatment, and 3) prevention.

The research areas in the program segments include:

1. Perinatal

Develop and utilize large scale methods to study the relation between viral, bacterial, mycoplasma and protozoa infections in the perinatal period and birth defects, related abnormalities and pediatric neurological diseases. Investigate approaches to early diagnosis, treatment and prevention using combined laboratory and clinical studies.

2. Acute

Investigate agents which may be responsible for acute neurological diseases such as meningitis, encephalitis, Bells' Palsy, and tic douloureux as well as possible methods for rapid diagnosis, treatment and prevention.

3. Chronic

Study chronic neurological diseases such as multiple sclerosis, amytrophic lateral sclerosis, Parkinson's disease, subacute sclerosing panencephalitis, Alzheimer's and Pick's disease and epilepsy using combined tissue culture, immunological serological, electron microscopic and clinical approaches for possible infectious etiologies. Whenever possible explore methods for early diagnosis, treatment and prevention.

III. Section Activities

1. Immunochemistry and Clinical Investigations

A. Perinatal

The Section is responsible for the research and the analysis of Collaborative Study sera and data for infection in 60,000 pregnancies. The approaches being used now include: 1) Clinical infections - correlation with pregnancy outcomes, 2) Serological investigation of 4000 abnormals and 4000 controls, and 3) High IgM among 30,000 children as a method to identify infected children.

Additional studies include high risk children and infections in relation to infertility and abortions. A new study has been initiated on virus C-particles in placentas and fetal tissues. Vaccines for herpes and cytomegaloviruses are under development. Studies of alpha-fetoprotein for diagnosis of neural tube defects are in progress. Experimental animal studies with cyclopamine are being conducted with the Section on Experimental Pathology.

B. Acute

New limulus tests and counter immunoelectrophoresis are being studied for the rapid diagnosis of meningitis. Drug treatment studies for herpes viruses are in early phases. Human volunteer investigations of rubeola and rubella vaccines are being conducted at the Petersburg Federal Reformatory. Defective delinquents are being tested for herpes antibodies.

C. Chronic

Clinical studies of the treatment of SSPE with transfer factor are being concluded and a new drug study will be started. Special genetic (HLA) and serological investigations of MS and Parkinson's disease patients are in progress.

HLA studies of SSPE patients have been reported and are now being extended. Serological studies of ALS patients are in progress. The levels of globulins and enzymes in the spinal fluid of MS patients have been determined. A cellular fusion factor is under investigation.

New radioimmune methods for antibody assays are under development. Viral chemotherapy with several new drugs are in early stages.

2. Section on Virology and Bacteriology

A. Perinatal

This section is conducting studies of the frequency of perinatal, amniotic, ovarian, and cervical infections. In addition, a study of neonatal deaths associated with infections is being conducted.

B. Acute

Chemotherapy of Flu, Reo and other agents are under investigation with the Section on Experimental Pathology. The frequency of hepatitis in CNS damaged children is being studied at the Lynchburg Training School.

C. Chronic

Mechanisms of cellular immunity to various viruses are being tested using patient material from individuals with MS, SSPE and other neurological diseases.

The pathogenesis of PML (SV_{40} like) agents are under study with the Section on Experimental Pathology. Also possible virus etiologies of MS are under investigation using a variety of techniques.

The immune response of monkeys to herpes virus is being studied. New antiviral drugs are being tested in vitro and in experimental animals with chronic infections.

Electron microscopic studies of MS, SSPE, visna and scrapie are under intensive investigations using immune peroxidase and freeze fracturing.

3. Section on Experimental Pathology

A. Perinatal

This section is conducting extensive studies of experimental monkeys which develop CNS and other damage when infected in utero. Current agents include mumps, flu, Reo and VEE viruses. Combined effects with infections and chemical carcinogens are also being studied. The value of new chemotherapeutic materials is under investigation.

B. Acute

Transmission studies of hepatitis A and B as well as Reo viruses are under investigations. Most of the agents can result in chronic infections. Treatment studies for CNS herpes are in progress.

C. Chronic

Intrauterine inoculation of pregnant monkeys with MS and other tissues is being continued by the Section. Chronic infection with SHF virus is being studied in detail. The role of herpes in cancer is being studied in collaboration with the National Cancer Institute.

IV. Findings

1. Perinatal

A. Influenza A Virus as a Teratogen in Pregnant Rhesus Monkeys

Experimental infection of pregnant rhesus monkeys at 100 days gestation lead to hydrocephalus in over 50% of the babies. These effects could be neutralized by specific antisera. These findings are particularly important in our understanding of the causes of hydrocephalus and caution our use of new live vaccines for Flu.

B. Hepatitis and Down's Syndrome

Hepatitis B was shown to cross the placenta and persist as a chronic infection in children. In addition, Down's syndrome patients became infected and retained the antigen for many years. The mechanism of persistance of this infection is important for our understanding of chronic virus diseases.

C. Interferon Response in Fetal Monkeys

Fetal monkeys infected in utero at various times in gestation were found to be unable to produce interferon in the early months of gestation. By mid-gestation, however, they were fully capable of making high levels of interferon. This deficiency in the immune response together with our previously reported findings for the inefficient antibody responses of the young fetus indicate that early in gestation this primate is particularly vulnerable to infection.

D. Cervical Cytomegalovirus Excretion

In this joint study with investigators at the University of Alabama, the excretion of CMV virus in pregnant and non-pregnant women was studied. It appears that early in pregnancy there is a suppression of CMS virus excretion, however later in pregnancy the virus appears more frequently (3-15%). Higher rates of infection are also found in non-pregnant females.

2. Acute

A. Characterization of a New Mycoplasma Species

A new mycoplasma species was isolated from Grivit monkeys. We have named this species Mycoplasma Moatsii and have reported on the characteristics of the species.

B. Hepatitis B Serotypes in an Institution for the Mentally Retarded

The frequency of hepatitis B infection is quite high in institutionalized individuals and in some groups antigen persists for many years. The serotypes found were those normally associated with chronic hepatitis in non-institutionalized individuals.

C. Computerized Primate Infection Retrieval System

To handle the large number of studies currently conducted with our primates, working in collaboration with the Office of Biometry, NINCDS, a computerized system was developed for all data storage and retrieval. This system is now being introduced for our routine use in primate studies.

D. Interferon Induction in Non-human Primates by a Nuclease Resistance Polyriboinosinic - Polyribocytidylic Acid Complex

This new material has proven to be a potent interferon stimulator in monkeys. It has direct clinical application for animal diseases.

E. A Further Miniaturized Serological Microtechnique

This new miniaturized method permits 100 serological tests with each milliliter of reagent or serum. This provides greatly increased savings in costly reagents and many tests can now be conducted on small amounts of sera.

3. Chronic

A. Availability of Serological Tests for the Diagnosis of SSPE

The use of spinal fluid rubeola CF tests now permits the rapid diagnosis of SSPE in almost all cases. This test alone makes brain biopsy unnecessary in almost every instance of SSPE.

B. SSPE - Destruction of Human Brain Cells by Complement Mediated Antibody

Collaborative studies with investigators at the Scripps Institute have shown that antibody from SSPE patients when combined with human complement is capable of destroying SSPE infected cells.

C. Immunoperoxidase Labeling of SSPE Virus in Acute Hamster Encephalitis

This study demonstrated the method for intracellular labeling of viral antigen in brain using the EM-immunoperoxidase method. Nuclear and cytoplasmic non-structural antigens were also demonstrated.

D. Unusual Nuclear Inclusions and Lengthy Clinical Causes of SSPE

In collaboration with investigators at the National Naval Medical Center, a patient with a lengthy clinical course was reported. These types of nuclear inclusions were found: 1) The typical paramyxovirus nucleocapsids 2) Dense strands and 3) Reticulum of dense bars. Virus maturation in brain was compared to *in vitro* maturation.

E. Cellular Immunity to Rubella in Congenital Rubella

Children with congenital rubella had reduced cellular immune responses to rubella when studied with lymphocytotoxic assay.

F. Monkey as a Model for Cervical Herpes Infection and Cancer of the Cervix.

Repeated cervical inoculations of Cebus monkeys for a period of 15 months or more have resulted in 1/3 of the animals showing persistant atypia in cervical cytology tests. These studies are being continued as a joint effort with the NCI. Newborn monkeys, born to these animals, have now shown congenital infection.

G. RAT Technique for Assay of Cellular Immunity

A new, safe, rapid method for transfer of radioiosotope labeled materials from a 96 well reaction plate was developed. This method is now being used routinely in our laboratory and will be available commercially shortly.

H. Cellular Immune Response to Herpes 1 in Recurrent Herpes Labialis

In collaboration with other investigators, it was found that lymphocyte blastogenesis was increased but cytotoxicity was decreased in patients with recurrent herpes labialis.

I. Multiple Sclerosis and Cell Mediated Immunity

Studies of lymphocyte migration inhibition gave partial confirmation to the report by Dr. Zabriski of the Rockefeller Institute that MS patients' lymphocytes fail to respond in a normal manner when exposed to measles virus.

CONTRACT NARRATIVE
Infectious Diseases Branch, IR, NINCDS
Fiscal Year 1975

THE UNIVERSITY OF WISCONSIN (NS42308)

Title: Study of the Use of Zonal Ultracentrifugation and Affinity Chromatography in the Etiology and Diagnosis of Multiple Sclerosis

Contractor's Project Director: Dr. Steven E. Kornguth

Current Annual Level: $113,888.00

Objectives: The basic objective of this contract is to investigate the possible etiology and diagnosis of MS. To gain information into these areas we desired to utilize the technique of zonal ultracentrifugation to purify and concentrate subcellular elements in MS brain tissue and utilize electronmicroscopy, affinity chromatography and biochemistry to identify antigens and/or antibody against subcellular particles. Hopefully, we will gain some insight into the pathogenic process and identity of possible etiologic agents.

Major Findings: During the first year of the contract, 3 MS brains have been fractionated by zonal ultracentrifugation, various fractions are being studied by electronmicroscopy, a number of affinity chromatography columns have been made. Normal globulin was used in an attempt to determine which type of column, coupling and uncoupling which should be used for the study of MS sera and spinal fluids. This has been determined and extraction of MS spinal fluid globulins is now in process. A manuscript has been prepared, submitted and accepted on the preservation of synaptic complexes of frozen MS brains.

Significance to the NINCDS Program and Biomedical Research: The goal of the NINCDS is to carry out planned directed research programs concerned with the diseases which damage the human nervous system. Multiple Sclerosis affects over 200,000 in the U.S. and many more throughout the world. Recent evidence suggests a possible infectious etiology for MS and these findings need to be investigated. The exceptional resolving capabilities of rate zonal ultracentrifugation and the use of affinity chromatography should help to identify possible etiologic agents.

Proposed Course of the Project: Samples from the rate zonal separation of MS brain tissue will be passed through affinity chromatography columns labeled with the gamma globulin of MS spinal fluid. Fractions will then be collected from these columns, materials pelleted and observed by electronmicroscopy and biochemical techniques.

CONTRACT NARRATIVE
Infectious Diseases Branch, IR, NINCDS
Fiscal Year 1975

THE UNIVERSITY OF CALIFORNIA, LOS ANGELES (NIH-NINCDS-69-5)

Title: Infections in Pregnancy and Infertility

Contractor's Project Director: Dr. Margaret Jones

Current Annual Level of Support: 27,281

Objectives: Four studies were included in this contract. One new study on rubella SSPE is to be added.

1. Effect of Viral Infections During Early Pregnancy

This is a study of 4,000 pregnancies. The study is unique in that pre-pregnant sera as well as sera obtained throughout pregnancy are available. Tests have been completed for rubella, Flu A, herpes, mumps, and cytomegaloviruses. Cord sera have been tested for IgM levels (NINCDS). Analysis has been concentrated in Flu A and mumps. The children are being reevaluated. Two controls are evaluated for each child.

2. Toxoplasmosis

A special study of 1,500 women registered early in pregnancy serial blood specimens have been tested for toxo antibody using the IHA method. A total of 200 abortuses have been tested for presence of the organism. Cord sera are being tested for toxo specific IgM (NINCDS). Children are being examined longitudinally. There were 42 women who had evidence of toxoplasmosis during pregnancy. Nine children have toxo specific antibody. The children and controls are being reevaluated.

3. Infertility Study

Eighty patients and eighty controls are being studied serologically for evidence of Herpes I, II and cytomegalovirus infections. Statistical analysis of patient-control pairs is nearing completion.

4. Cytomegalovirus Special Studies

Two thousand women registered in the first trimester of their pregnancies (1967-1971) have been studied. Serological tests have been performed. Urine, cervical swabs and throat swabs have been tested for CMV. Cord sera are being tested for IgM. Analysis is in progress. Children are being followed longitudinally. There were 27 positive cervical cultures, 2 urine cultures, and 26 children with specific IgM antibody. The children and controls are being reevaluated.

Major Findings:

1. Viral Infections During Early Pregnancy -

   Flu A: 113 women were found to have had Flu A.
   Approximately half of the available children have been reevaluated.

   Mumps: 30 women had mumps.
   Four children have been reevaluated. It is anticipated that 10 more will be available for retesting.

2. Toxoplasmosis -

   51 mothers had toxoplasmosis and 42 children had specific IgM antibody. For the children, 31 have been reevaluated and 7 more will be tested next year.

3. Cytomegalovirus -

   55 mothers had CMV infection. 10 children will be reevaluated this year and 10 next year.

Proposed Course of Project: The project will be extended at no additional cost for one year. This will permit the completion of reexaminations of children and reporting of results. A special study of rubella pregnancies will be conducted with approximately 100 rubella children. They will be tested for Rubella SSPE using serology and neurological examinations.

CONTRACT NARRATIVE
Infectious Diseases Branch, IR, NINCDS
Fiscal Year 1975

DEPARTMENT OF PEDIATRICS, UNIVERSITY OF TENNESSEE (PH43-68-17)

Title: Identification of Specific Antibodies in the Serum IgM Fraction of High Risk Infants

Contractor's Project Director: Dr. Sheldon Korones, M.D.

Current Annual Level: $23,451

Objective: The study is designed to assess the validity and clinical usefulness of specific tests for serum IgM for the identification of antibodies to rubella, cytomegalovirus, toxoplasmosis, herpesvirus and syphilis. When these diseases are identified as being present in the children, appropriate therapeutic approaches are used.

Major Findings: Approximately 2,305 infants have been tested to date. Serial blood specimens are collected twice each week from each study infant for the duration of the nursery stay. Venous blood obtained from infants with IgM concentration is in excess of 20 mgm percent in the first two weeks of life and 30 mgm percent between the third and fourth weeks. Virus cultures are obtained from all infants with high IgM levels. Clinical evaluation data is being maintained for correlation with the serologic findings.

Significance to the NINCDS Program and Biomedical Research: The detection of high risk infants with congenital infections is hampered by the lack of specific tests to identify these children. Recent studies have indicated that elevated IgM levels are present in at least 80 percent of children with congenital infection. The present studies will provide direct information as to the usefulness of specific IgM antibody determinations for five major infectious agents as a means of detecting not only the possible presence of congenital infection but the specific infection which requires treatment. New methods for the removal of IgG will be added to the IgM, FA procedures.

Proposed Course of the Project: The project is completing its third year. We anticipate two additional years of study during which a total of 5000 infants will be examined. This year specific FA test will be done at the nursery for syphilis, toxoplasmosis and cytomegalovirus using new kits now available.

CONTRACT NARRATIVE
Infectious Diseases Branch, IR, NINCDS
Fiscal Year 1975

SCRIPPS CLINIC AND RESEARCH FOUNDATION (N01-NS-4-2309)

Title: Detection of Viral Genomes in Human Neurological Diseases

Contractor's Project Director: Dr. William J. Meinke

Current Annual Level: $63,387.00

Objectives: Contractor will attempt to demonstrate the presence or absence of viral genes in cells derived from patients with chronic neurological diseases, principally multiple sclerosis, amyotrophic lateral sclerosis, and Alzheimer's disease. The experimental approach is to analyze cellular nucleic acids derived from Central Nervous System tissues by extremely sensitive molecular DNA-DNA and DNA-RNA hybridization techniques.

Major Findings: Contractor recently developed an in vitro procedure for labeling viral DNA. This is accomplished by a "nick-repair" enzymatic reaction. Viral DNA is "nicked" with dioxyribonuclease and then repaired using E coli DNA polymerase. Specific activities greater than $2x10^7$ counts per minute per microgram have been obtained. This is very important since the high specific activities produced with the viral probes require less cellular DNA for each experiment. Viral probes for herpes virus type 1 and type 2 have been prepared and are presently being used on extracted material.

Significance to the NINCDS Program and Biomedical Research: Chronic neurological diseases may be caused by the incorporation of viral nucleic acids into brain cells and thereby control the production of cell surface changes without the expression of complete virus. Co-cultivation techniques and other methods of isolating virus may not be possible. With the use of radioactive probes high sensitivities for the detection of viral nucleic acids is now possible.

Proposed Course of the Project: Additional DNA viral probes will be prepared using the "nick-repair" enzymatic reaction. DNA will be extracted from other neurological diseases and the experimental approach as previously described will be utilized. A supplement has been proposed to be added to this contract and with additional funding, RNA probes of various viruses can be produce and also studied.

Serial No. Z01 NS 00404-10 ID
1. Infectious Diseases Branch
2. Section on Immunochemistry and Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunology of Perinatal Infections

Previous Serial Number: Same

Principal Investigators: Dr. John L. Sever, IDB, NINCDS
Dr. David A. Fuccillo, IDB, NINCDS
Mrs. Anita Ley, IDB, NINCDS
Mrs. Renee Traub, IDB, NINCDS
Mrs. Mary Ruth Gilkeson, IDB, NINCDS
Mrs. Flora Moder, IDB, NINCDS
Dr. Jonas Ellenberg, OD, NINCDS
Mrs. Eudora Beadle, OD, NINCDS

Other Investigators: Dr. Gabriel Castellano, MRA
Dr. Janet Hardy, Baltimore, Md.
Dr. Paul Terasaki, UCLA, Los Angeles, California

Cooperating Units: Collaborating Institutions in the Perinatal Research Study
Laboratory of Infectious Diseases, NIAID
NICHD
Johns Hopkins University Medical School

Man Years:

Total	13.5
Professional:	5.5
Other:	8

Project Description:

Objectives: The purposes of this study is to determine insofar as possible the role of infections and immunity in the production of abnormal pregnancy outcomes. To accomplish this, 12 collaborating institutions in the Perinatal Research Study plus two special cooperating groups in separate studies have been obtaining specimens of blood and tissue throughout pregnancy, at delivery, post partum, and at set intervals thereafter. These sera are tested to determine the antibody responses of the patients during pregnancy and post partum and then to relate this serological information to the clinical data for the pregnancy and child. In addition, serum specimens from the children were obtained at one year of age from 10,000 study pregnancies. Sera, throat swabs, and urine specimens were also obtained from approximately

5,000 pregnancies. Placental specimens were obtained from 2,500 pregnancies. In special cases when congenital infection is suspected on the basis of clinical or laboratory findings, throat swabs and blood specimens are obtained from the children. Immunoglobulin determinations are performed with the cord blood specimens from the children and specific antibody determinations are also made with these specimens.

Methods Employed: To accomplish this program, blood specimens were obtained from pregnant women at set intervals throughout pregnancy and post partum. Completeness of the sets of sera is determined at the Serum Center of the Infectious Diseases Branch. Data for the 58,000 patients in the Collaborative Perinatal Research Study show that specimens are available from 94.2% of the patients. An average of 5 blood specimens is available for each patient. Each specimen consists of 4 vials with 3 ml of serum in each. For this study then, there are approximately 300,000 serum specimens and almost a million and a half vials of sera. There are an additional 5,000 patients studied to date at the Kaiser Hospital in Los Angeles and approximately 3,000 under study at the Johns Hopkins Medical School in Baltimore, Maryland. All specimens are stored at -20°C until tested and complete filing record concerning basic patient information and the status of the serum available is maintained through a computer system by the Serum Center of the Branch.

In addition to the serum specimens, serial urine and throat specimens were also obtained on a large majority of the patients in the two special studies. These are being studied for direct virus isolation along with swabs obtained from the children at the time of birth.

To date, approximately 58 publications have resulted from the analysis of the data from these studies. The serological method most frequently employed is the complement fixation test with the use of viral antigens. The test is very versatile and can be performed rapidly and provides broad coverage for a great many of the more than 130 viruses which are known to be of importance to man. Antigens were prepared for most of these viruses and tests of specificity were conducted with animal sera. In addition to the complement fixation method, hemagglutination inhibition tests are used for many viral serological determinations when greater specificity is needed, neutralization methods are employed. Indirect fluorescence is also used for some of the studies. Virus isolation is conducted with tissue culture of inoculation of experimental animals.

All tests are reproduced completely and a minimum of 95% agreement within two-fold variation is required. All sera showing significant changes in antibody, together with any sera which did not reproduce are tested the third time. We are now completing the study of reported viral, bacterial, and protozoal infections in pregnant women in the study. Serological tests are used to document these reports. The data is then correlated with the pediatric findings. Approximately 2,500 cases of reported viral infections, 3,000 bacterial infections and several hundred protozoal infections, are under investigation. Clinical data is being abstracted, serological tests are being performed in order to document these infections. There are also approximately 1,200 patients identified with a positive serology for syphilis. These are being studied in detail.

A second approach involves a large scale study designed to investigate infection and immunity in relation to 4,000 normal children in the study and 4,000 matched controls. The print-out of abnormal patients has been obtained from the Collaborative Perinatal Research Study (PRB) and this is being reviewed in detail by nurses and physicians from the IDB for more complete information.

From study records, the specific abnormalities under study include abortions, stillbirths, cataracts, congenital heart disease, neonatal deaths, low birthweight (1,000-1,500, 1,500-2,000 grams), IQ below 50, IQ 50-69, enlarged liver, malformations, retarded gross motor development, retarded fine motor development, hearing deficit in both ears, visual impairment, cranial or peripheral nerve damage, cerebral palsy, delayed motor development, hypotonia with poor deep tendon reflexes, non-febrile seizures, dyskinesia and ataxia, hearing deficit in one ear, and elevated bilirubin. The specimens from the mothers of these children and from the children themselves along with carefully matched controls are being studied for antibody to 11 antigens. These antigens include influenza A, rubeola, rubella, mumps, Coxsackie B$_3$, Coxsackie B$_4$ varicella, toxoplasmosis, cytomegalovirus, herpes type I, and herpes type II. All of these agents are known or suspected to be responsible for damage in the perinatal period. All laboratory work is being performed under code. The data is being analyzed by Mrs. Gilkeson and Dr. Ellenberg. A second phase of this study will involve 4 additional antigens.

The third approach is to identify the children with elevated IgM levels in the newborn period and then to correlate these findings with pregnancy outcome, clinical performance of the child, and specific serological tests for IgM antibody. Almost 32,000 cord sera have been tested for IgM antibody and approximately 2,000 show elevated levels. These are now being studied in detail.

Major Findings: 1. Maternal Clinical Infections and Pregnancy Outcomes - The frequency of clinical viral, bacterial, fungal, and parasitic infections was determined for the 58,828 pregnancies in the Perinatal Research Study. There were 8,180 clinically infected pregnancies (13.9%). The infections included: viral 3,401; bacterial 4,539; fungal 102, and parasitic 138. Influenza "flu"-like illness occurred at the rate of 270 cases/10,000. Specific infections such as mumps, rubella, chickenpox/zoster and measles occurred at rates of 2 to 19 cases per 10,000 pregnancies. Bacterial infections of the kidneys - ureteral - bladder were very frequent (336/10,000 pregnancies). Gonorrhea occurred at a rate of 32/10,000 pregnancies. Pinworm infections were the most frequent of the parasitic infections (12/10,000).

2. Serological Studies of Perinatal Infections and Pregnancy Outcome - Nine abnormal pregnancy outcomes were studied: abortions, stillbirths, cataracts, microcephaly, congenital hearts, neonatal deaths, low birthweight (1001-1500 gms), low birthweight (1501-2000 gms) and low IQ (≤ 50). Eleven antigens were used. More than 100,000 antibody titrations were performed. Several leads have already appeared: 1) Herpes virus II in relation to microcephaly and abortions; 2) toxoplasma gondii in relation to stillbirths, neonatal deaths, and low birthweight; 3) Coxsackie B_3, CMV, and mumps in relation to certain forms of congenital heart disease and mental retardation.

3. Cord IgM Levels and Specific IgM for the Detection of Congenital Infections and Fetal Damage. Neonatal serum IgM levels are usually elevated in congenital infections. Cord blood samples 31,926 children had high IgM ($\geq$ 20 mgm %). Specific tests for rubella, cytomegaloviruses and syphilis are now in progress. There were 8 children with definite congenital syphilis. One was a stillbirth. There are 14 cases of suspected congenital syphilis.

Cytotoxic (HLA) antibodies present in the serum of women with various abnormal pregnancy outcomes and matched controls were studied with Dr. Paul Terasaki, UCLA. A total of 3.5% of women who were pregnant for the first time had antibodies, however, this increased 21 to 61 percent when the gravidities were three or more. These studies indicate that antifetal tissue antibodies are present in these pregnancies.

Collaborating Studies: A primary deficit in the data from the Collaborative Perinatal Research Study has long been recognized as the late registration of the study's patients. Since only 20% of the patients registered during the first trimester of pregnancy, it is impossible to document adequately the infectious disease experience of the patients during the first trimester. To provide data on the first trimester of pregnancy and high-risk pregnancies, an additional cooperative study was initiated with the Infectious Diseases Branch. This is a study of viral infections in pregnancy with the University of California, Los Angeles.

A second study was initiated at the Kaiser Hospital, Los Angeles, California, to study infection in relation to infertility. Approximately 50 women are being studied each year. In each case sera, throat swabs, cervical swabs and endometrial biopsy specimens are obtained and sent to the IDB laboratories for detailed study.

A second deficit of the Perinatal Research Study has been the lack of serial blood and tissue specimens from children with known congenital infection. For this reason, a special investigation was initiated with Dr. Janet Hardy at the Johns Hopkins Medical School to provide longitudinal surveillance of children with congenital infections. In this study we determine the clinical course, as well as the antibody levels, immunologic responses, and shedding of infectious agents in these children. A new study of HLA antibody in pregnant women and its effect on the baby was initiated with Dr. Paul Terasaki, UCLA.

Significance of the Program to the Institute: The use of micro-serological techniques for a large group of new viruses provides an opportunity to investigate the disease caused by viruses which are either difficult to isolate or resistant to evaluation because the clinical effects are delayed until a long time after the infection has subsided. In addition, the availability of new immunologic techniques provides the unique opportunity to detect immunologic deficits and to determine the presence of intrauterine infections on the basis of immunologic response. This data can then be correlated and analyzed as in relation to the possible causes of birth defects. The application of this type of analysis has provided valuable information on the epidemiology of virus infections in relation to abnormal pregnancy outcomes and is constantly giving us new insights into the causes of damage to the central nervous system and possible means of prevention of this and other damage to the developing fetus and newborn.

Proposed Course of the Project: The combined immunologic virologic program will continue during the next year. During that time we will complete the remaining tests for the first phase of the serological studies. Phase two testing will then be initiated using 4 new antigens, including EB and hepatitis.

The three approaches which are being emphasized now include:

1. Completion of the correlation of clinically reported infections in pregnancy with serological findings for the pregnancy, immunologic determinations, and pregnancy outcome. These studies should be completed for the most part in the next fiscal year.

2. A special commitment to perform serological tests on 4,000 abnormal pregnancies and 4,000 matched controls using 11 antigens. The abnormal children have been identified and the laboratory is now approximately 90% of the way through the testing.

3. Special testing of IgM levels from 32,000 cord sera from children in the Collaborative Study and in the cooperative studies. This work provides an index for identifying children with possible congenital infections so that more specific testing can then proceed. These investigations are now well under way and will be continued during the next year. Approximately 2,000 cord sera have high IgM levels. These are being tested for specific antibody to toxoplasma, cytomegalovirus, and syphilis.

New rapid methods for determining IgM levels and specific IgM antibody will be investigated further. Procedure for removing IgG will be studied further.

Keyword Descriptors: perinatal infections, collaborative perinatal study, HLA antibody.

Honors and Awards: None

Publications:

Sever, J. L.: Infectious Agents as Teratogens. Published in Methods for Detection of Environmental Agents that Produce Congenital Defects. Proceedings of the Guadeloupe Conference sponsored by the Institute de la vie (In Press).

Sever, J. L., Fuccillo, D. A., Bowes, W. A.: Prevention of Fetal and Perinatal Diseases Environmental Factors: Infection and Immunizations. Prevention of Embryonic Fetal and Perinatal Disease (In Press).

Serial No. Z01 NS 00835-15 ID
1. Infectious Diseases Branch
2. Section on Immunochemistry and Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Clinical Investigations with Human Volunteers and Other Populations Using Viruses, Vaccines, and Chemotherapeutic Agents

Previous Serial Number: Same

Principal Investigators: Dr. John L. Sever, IDB, NINCDS
Dr. Jerome Kurent, IDB, NINCDS

Other Investigators: Dr. David A. Fuccillo, IDB, NINCDS
Dr. Michael Blaese, NCI
Dr. Paul McCallin, Kaiser Hospital, Hawaii

Cooperating Units: Bureau of Prisons, Dept. of Justice
Petersburg Federal Reformatory

Man Years:

Total:	1
Professional:	.5
Other:	.5

Project Description:

Objectives: To study new prophylactic and therapeutic materials for the prevention and control of infectious diseases. To study the safety, antigenicity, communicability, and immunogenicity of candidate vaccines.

Methods Employed: Human volunteer studies are conducted in collaboration with the Federal Bureau of Prisons. These studies are reviewed and approved by the Clinical Research Committee and the Medical Board of the National Institutes of Health and the Medical Review Board of the Federal Bureau of Prisons.

Vaccines and chemotherapeutic agents which appear to warrant further investigations are then utilized in clinical studies of other special patient groups. In addition, clinical studies of epidemic are investigated as a means of identifying the role of infectious agents in the production of perinatal and pediatric disease states.

Major Findings: The immune response of volunteers with rubeola and rubella vaccines have been compared with the immune responses of patients with SSPE and MS. For SSPE there appears to be a serum inhibitor which blocks cellular immunity to measles The nature of the inhibitor is being investigated.

Transfer factor was given to 8 patients with SSPE. These patients have been followed for 3 years. It appears that some of the patients have not had the expected rapid progression of disease. These studies will be extended.

Significance of the Program to the Institute: Volunteers studied provide the basic data necessary to evaluate potential chemotherapeutic agents and vaccines. They also provide invaluable information on the course of infection in man. Utilization of this information then in clinical studies provides the necessary bridge to the implementation of therapeutic approaches which originated in the laboratory, are brought through the volunteer studies and then finally are taken to patient population.

Proposed Course of the Project: The additional studies will be performed utilizing rubeola and vaccines to determine antibody and cellular immune responses. Rubeola antibody and cellular immunity in SSPE patients will be studied in detail. Clinical studies will continue on utilization of various drugs for the prevention of treatment of perinatal, acute and chronic infections in man. The drug Disodiumphosacetic acid is being considered for treatment of herpes and pox viruses. The drug Ribavirin is being studied with rubeola, rubella and SHF.

Keyword Descriptors: Acute infections, immune responses and human volunteers.

Honors and Awards: None

Publications:

Sever, John L., Krebs, Helen; Ley, Anita; Barbosa, Luiz H.; and Rubinstein, David: Diagnosis of Subacute Panencephalitis - The Value and Availability of Measles Antibody Determinations. JAMA, Vol. 228, No. 5, 1974.

Serial No. Z01 NS 01270-11 ID
1. Infectious Diseases Branch
2. Section on Immunochemistry and Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Toxoplasmosis: Serological and Clinical Studies

Previous Serial Number: Same

Principal Investigator: Dr. John L. Sever, IDB, NINCDS

Other Investigators: Dr. Joseph S. Drage, PRB, NINCDS

Cooperating Units: Section on Pediatric Neurology, PRB, NINCDS

Man Years:

Total:	0.4
Professional:	0.2
Others:	0.2

Project Description:

Objectives: This study relates to antibody titer in pregnant women to abnormal pregnancy outcomes. A total of 23,000 women have now been studied.

Methods Employed: The computer analysis of multiple variables relating toxoplasmosis in pregnancy outcome has been used. The serologic data is being correlated with pregnancy outcomes.

Major Findings: Within the group of 47 patients with high titers or significant increases in antibody titers, 5 were found to have definite toxoplasmosis and 10 were suspected of having possible toxoplasmosis. There were increased rates of stillbirths and neonatal deaths. The acquisition of antibody to toxoplasmosis was strongly correlated with age and race.

Proposed Course of the Project: Publication of complete analysis will be completed this year. Data for 7-8 year IQ levels will be analyzed using matched controls.

Keyword Descriptors: Perinatal Infections, Toxoplasmosis

Honors and Awards: None

Publications: None

Serial No. Z01 NS 01503-09 ID
1. Infectious Diseases Branch
2. Section on Immunochemistry and Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Epidemiologic Studies of Perinatal Infections

Previous Serial Number: Same

Principal Investigators: Dr. John L. Sever, IDB, NINCDS
Dr. Jerome E. Kurent, IDB, NINCDS
Mrs. Dorothy Edmonds, IDB, NINCDS
Mrs. Anita C. Ley, IDB, NINCDS
Mrs. Flora Moder, IDB, NINCDS

Other Investigators: Dr. Jonas Ellenberg, OB, NINCDS

Cooperating Units: Collaborative Perinatal Research Study
Kaiser Hospital, Los Angeles, California
Kaiser Hospital, Hawaii
Kaiser Hospital, Oakland, California
Dr. Thomas Waldmann, NCI
Microbiological Associates, Bethesda, Md.

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives: Utilize information from collaborative and cooperative studies to identify pregnancies complicated by maternal infections or infections in childhood. To further delineate these cases by serological testing and to determine the outcomes of these pregnancies in relation to the infections which have been defined.

Methods Employed: Primary material utilized for these studies are the clinical records, serum and tissue specimens obtained during pregnancy and the pediatric period of observation. Serologic data developed by the immunology laboratories is correlated with the clinical information from the collaborating groups. Data is analyzed in cooperation with the Office of Biometry, NINCDS.

Major Findings: At least a dozen viruses have been identified as being of importance in the perinatal period. The specific rates of the occurrence of these infections appear to be excess of 5 percent of the total pregnancies. As many as 10 percent of the children with microcephaly and mental retardation may be associated with these perinatal infections.

Maternal hepatitis at term frequently results in transmission of the virus to the fetus and chronic fetal infection then follows. C-particles are found in human placentas indicating vertical transmission of this virus.

Significance of the Program to the Institute: The epidemiologic studies which are possible because of the large numbers of patients in the collaborative and cooperating studies provide the necessary data for these types of investigations. Special studies are initiated in populations where high frequencies of infection or abnormal pregnancy outcomes have been noted.

Proposed Course of the Project: New Phase II studies will be initiated using EB virus, hepatitis and two Coxsackie B viruses. These antigens will be employed with the 4000 abnormals and 4000 control sera from the Collaborative Study.

Keyword Descriptors: Perinatal infections, EB virus, hepatitis, and Coxsackie B virus.

Honors and Awards: None

Publications: None

Serial No. Z01 NS 01992-04 ID
1. Infectious Diseases Branch
2. Section on Immunochemistry and Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: High Risk Pregnancies and Perinatal Infections

Previous Serial Number: Same

Principal Investigators: Dr. John L. Sever, IDB, NINCDS
Dr. David A. Fuccillo, IDB, NINCDS
Dr. Luiz H. Barbosa, IDB, NINCDS
Dr. Sheldon Korones, University of Tennessee
Dr. Margaret Jones, UCLA-Kaiser Hospital, L.A.

Other Investigators: Dr. Gabriel Castellano, MBA

Cooperating Units: University of Tennessee
UCLA

Man Years:

Total:	1
Professional:	0.5
Other	0.5

Project Description:

Objectives: The purpose of this investigation is to study high risk pregnancies in relation to perinatal infections. To accomplish this, a special study was developed at the newborn nursery at the University of Tennessee. This is a high risk nursery where infants from the Memphis Metropolitan Area are brought for special newborn care and procedures. Serial blood specimens are obtained and tested for immunoglobulin levels as a means of identifying those children with possible intrauterine infection. In addition, other laboratory tests and specimens are obtained to further define the infections which may be present. This study focuses on a group of infants which is most likely to have difficulties in the newborn period and is most frequently involved with infections. In a second study at the Kaiser Hospital, Los Angeles, we are investigating the role of infection in abortions, infertility, and CNS disease in newborns.

Methods Employed: For the study at the University of Tennessee, serial heel prick blood specimens are obtained during the first weeks of life. These specimens are tested for IgM levels using radial immunodiffusion techniques. Infants showing elevated IgM levels are then further tested using venous blood specimens, throat swabs, anal swabs and other tissue specimens. These latter specimens are forwarded to the laboratories of the Infectious Diseases Branch for study. Virus isolation procedures and specific antibody tests are used. The data is then analyzed in relation to the clinical findings in the child and treatment is instituted wherever appropriate.

For the study at the Kaiser Hospital, 4,000 pregnant women have been studied to determine the frequency of infection and the effects of the infections on the children. Additional studies of cytomegalovirus by direct isolation of the virus are being conducted. A special study of 100 women attending an infertility clinic is in progress.

Major Findings: At the University of Tennessee approximately 2,000 children have been studied this year. We find that an unusually high rate of IgM elevation in this population exists. Investigations are in progress to define the specific organisms which may be responsible for the congenital infections. At the Kaiser study, higher rates of infection with toxoplasmosis and cytomegalovirus have been found among the women in the infertility groups.

The Significance of the Program to the Institute: In our effort to identify the causes of neurological and other damage to the developing child, these populations provide unique opportunities of studying high risk pregnancies and thus developing a great deal of information with a minimal amount of study and testing. We are focusing in on a population which is at high risk for congenital infections.

Proposed Course of the Project: The study at the University of Tennessee will continue during which a total of 5,000 patients will be studied. Combined serological and virus isolation techniques will be used. At the Kaiser-UCLA study, analysis of data will be completed next year. A new study of Rubella SSPE will be conducted to determine the frequency of this disease.

Keyword Descriptors:

Perinatal infections, high risk pregnancies, IgM

Honors and Awards: None

Publications: None

Serial No. Z01 NS 01993-04 ID
1. Infectious Diseases Branch
2. Section on Immunochemistry and Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Amniotic Fluid, Placentas, Fetal Infection, Antibody and Infection

Previous Serial Number: Same

Principal Investigators: Dr. John L. Sever, IDB, NINCDS

Other Investigators: Dr. David Madden, IDB, NINCDS
Dr. David Fuccillo, IDB, NINCDS
Dr. Geoffrey Altshuler, U. of Cincinnati
Dr. Mina Lee Vernon, MBA

Cooperating Units: D.C. General Hospital, Washington, D.C.
Children's Hospital, University of Cincinnati
Microbiological Associates

Man Years:

Total:	1
Professional:	0.5
Other:	0.5

Project Description:

Objectives: This is an investigation of amniotic fluid obtained at therapeutic abortion or amniocentesis to determine immunoglobulin levels and to attempt to isolate infectious agents. We are also investigating placental mycoplasma infections and their effect on birthweight. A new study of C particles in placentas and fetal tissues has been stated.

Methods Employed: Amniotic fluid specimens were obtained at amniocentesis for therapeutic abortions or serial determinations of bilirubin levels. Specimens are immediately brought to the laboratory for IgM, IgG and IgA determinations using the radial diffusion method and counter current electrophoresis. Aliquots of the specimens were also given to our other laboratories for virus isolation and mycoplasma isolation. Serum specimens are available from the mothers for subsequent antibody determinations. Placental specimens will be obtained for mycoplasma, isolation and histological studies.

Major Findings: A number of fetal tissue specimens have shown mycoplasma. Infection of the fetus with mycoplasma was found in 70% of the births. We have not found a correlation with the agentsand low birthweights. C particles ("Viruses") have been found in placental tissues. The possible spread of the infection to the child is under study.

Significance of the Program to the Institute: This is the first study of intrauterine immunoglobulin levels and the first attempt at isolation of infectious agents from the uterus. Previous studies have dealt with specimens obtained at term. This data is confusing both in terms of antibody levels and the presence of microorganisms since vaginal contamination can and does occur.

Proposed Course of the Project: We are in the process of completing the mycoplasma data for reporting during this next fiscal year. The presence of C particles in many placentas suggests that the tissue is infected in utero. We will determine if fetal infection is also present by the study of abortuses, stillbirths, and neonatal deaths.

Keyword Descriptors:

Perinatal infections, amniotic fluid, placentas, fetal tissues

Honors and Awards: None

Publications: None

Serial No. [illegible]994-04 ID
1. Infectious Diseases Branch
2. Section on Immunochemistry and Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Clinical Studies of Chronic Infections of the Central Nervous System

Previous Serial Number: Same

Principal Investigators:
Dr. John L. Sever, IDB, NINCDS
Dr. David A. Fuccillo, IDB, NINCDS
Dr. Jonas Ellenberg, Office of Biometry, NINCDS
Dr. Ronald Kartzinel, NINCDS

Other Investigators:
Dr. J. T. Jabbour, Memphis, Tennessee
Dr. George Schumacher, Vermont
Dr. Jacob Brody, EB, NINCDS
Dr. Milton Alter, Minneapolis, Minn.
Dr. John Kurtzke, VA Hospital, Wash., D. C.
Dr. Luiz H. Barbosa, IDB, NINCDS
Miss Helen Krebs, R.N., IDB, NINCDS

Cooperating Units:
University of Tennessee
Veterans Administration Hospital, Washington, D. C.
University of Vermont
University of Minnesota

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives: Clinical laboratory studies are being conducted on chronic infections of the central nervous system. During this year, the investigations have primarily centered on subacute sclerosing panencephalitis, multiple sclerosis, amyotrophic lateral sclerosis and Parkinson's disease. These studies have epidemiological, serological and therapeutic components. They involve collaboration of a number of groups through the United States.

Methods Employed: A registry for subacute sclerosing panencephalitis was initiated as a joint effort with Dr. J. T. Jabbour at the University of Tennessee. This registry now has data for more than 400 cases of SSPE. The reporting of patients is being continued to determine if there will be a change in the number of cases per year related to the widespread use of measles vaccine. This investigation has provided us with opportunities to study unusual patients such as one individual with hypogammaglobulinemia, as well as SSPE in one of two identical twins. Additional studies have been conducted on the epidemiology of multiple sclerosis. Serum and spinal fluid specimens are being tested for antibody to a variety of viruses. Similar studies of multiple sclerosis, amyotrophic lateral sclerosis and Parkinson's disease are in progress.

Major Findings: Analysis of the data for 280 patients with SSPE has brought out a number of new findings: 1) The "incubation" period between measles and onset of disease is 6.5 years ± 2.5 years. 2) This interval is suprising-constant for all ages of measles (1 year through 6 years). 3) The administration of measles vaccine after measles did not significantly change this interval. 4) Subacute sclerosing panencephalitis (SSPE) is rarely seen in Blacks in this study population although most patients come from rural South United States.

Patients with multiple sclerosis are being tested for cellular immunity in relation to measles and other viruses. Patients with Parkinson's disease are being tested for antibody to 11 viruses.

Significance of the Program to the Institute: Clinical studies of SSPE, MS ALS and Parkinson's disease and other chronic infections of the central nervous system permit direct investigation of the possible causes of these diseases, and provide us with an opportunity to study unique "experiments of nature" which often provide very valuable insight into the diseases process. These studies are designed to take advantage of both the epidemiology as well as the direct laboratory approaches to the problems of chronic infections of the CNS.

Proposed Course of the Project: We plan to continue this SSPE registry to determine the effect of the widespread measles immunization program, which has been in effect in the United States for more than 10 years. If the vaccine influences the rate of SSPE, this change should occur in the next few years.

Keyword Descriptors: Chronic infections, tissue culture, serology, and treatment.

Honors and Awards: None

Publications:

Sever, John L.: Summary of MS Conference. Neurology (In Press)

Serial No. Z01 NS 02038-03 ID
1. Infectious Diseases Branch
2. Section on Immunochemistry & Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Combined Clinical Viral and Immunological Investigations of Chronic and Subacute Disorders of the Central Nervous System

Previous Serial Number: Same

Principal Investigators:
Dr. Jerome Kurent, IDB, NINCDS
Dr. Luiz H. Barbosa, IDB, NINCDS
Dr. John L. Sever, M.D., Ph.D., IDB, NINCDS
Dr. David A. Fuccillo, Ph.D., IDB, NINCDS

Other Investigators:
Mrs. Anita Ley, IDB, NINCDS
Mrs. Flora Moder, IDB, NINCDS

Cooperating Units:
Medical Neurology Branch, NINCDS
 Dr. King Engel
University of Tennessee, Pediatric Neurology
 Dr. J. T. Jabbour
University of Vermont, Dept. of Neurology
 Dr. George Schumacher
Patuxent Institution, Jessup, Md.
 Dr. Sigmund Manne
UCLA
 Dr. Paul Terasaki

Man Years:

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description:

Objectives: To determine whether serological evidence exists to indicate that viral infection may be related to the pathogenesis of amyotrophic lateral sclerosis (ALS). To determine if any association exists between SSPE and HL-A histocompatibility antigens. To determine if defective delinquents have an increased frequency of antibody to herpes virus hominis.

Methods Employed: 1) Seroepidemiology: Serum and CSF specimens from ALS patients and neurologic controls will be studied for the presence of antibody to selected viruses. The presence of serum and CSF viral antibodies shall be determined by one of three methods depending upon the specific virus. These shall include indirect hemagglutination, complement fixation, and serum neutralization. Antigens shall include polio virus types I, II, and III; varicella-zoster; herpes Type I and II; cytomegalovirus; mumps; rubeola; rubella; influenza A, coxsackie B3 and B4.

2) Tissue Culture: Muscle biopsies from ALS patients were studied for the possible presence of viral antigen. Two of approximately ten muscle biopsies obtained from as many patients demonstrated fluorescence by the indirect immunofluorescent technique when treated with their own serum. This occurred only in first or second passage cultures. CPE did not occur with co-cultivation.

3) HL-A antigens and SSPE. Peripheral lymphocytes from 36 SSPE patients have been typed for HL-A antigens. A significant increase in the frequency of antigen W-29 was demonstrated when compared to normal blood donors.

Major Findings: ALS findings described in tissue culture above. An increased frequency of HL-A antigen W-29 has been demonstrated in a pilot study of SSPE patients. Serological investigation of defective delinquents did not demonstrate an increased frequency of herpes antibody when compared to non-defective delinquents.

Significance of the Program to the Institute: The search for viral etiologies of chronic and subacute human CNS disease by means of immunologic and virologic techniques is in keeping with the major objectives of the Infectious Diseases Branch. The demonstration of viral etiologies for CNS disorders would provide the rationale for its prevention and treatment, as well as to set the stage for an understanding of the pathogenesis of chronic neurological disease.

A positive association between HL-A antigen W-29 and SSPE might indicate that the presence of this antigen may be a risk factor in the development of SSPE. It would be consistent with the concept that host-determined factors may be important in the pathogenesis of SSPE.

Proposed Course of the Project: Serological studies of serum and CSF from ALS patients and neurologic controls are now in progress. HL-A and SSPE study will be terminated. Defective delinquent study will be terminated.

Keyword Descriptors: Amyotrophic lateral sclerosis, SSPE, defective delinquents, viral serological investigation, HL-A.

Honors and Awards: None

Publications: Fuccillo, David A., Kurent, Jerome E., Sever, John L.: Slow Virus Diseases. Annual Review of Microbiology 28: 231-264, 1974.

Kurent, Jerome E., Sever, John L.: Pathogenesis of Intrauterine Infections of the Brain. In Biology of Brain Dysfunction Vol. 3, Plenum Press. Ed. G.E. Gaull, 1975.

Kurent, Jerome E., Sever, John L.: Infectious Diseases and the Prevention of Mental Retardation. To be published in Infectious Disease and the Prevention of Mental Retardation and Genetic Disease, (Ed) A. Milunsky, W.B. Saunders Co., in press 1975.

Serial No. Z01 NS 02039-03 ID
1. Infectious Diseases Branch
2. Section on Immunochemistry & Clinical Investigations

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Blighted Potatoes and Birth Defects

Previous Serial Number: Same

Principal Investigators: Dr. Jerome E. Kurent, IDB, NINCDS
Dr. John L. Sever, IDB, NINCDS
Dr. William T. London, IDB, NINCDS

Other Investigators: Dr. Ken Deahl

Cooperating Units: Dr. Steve Sinden
Dr. Ramon Webb
Plant Genetics and Germ Plasm Institute
Vegetable Laboratory
Range I Agricultural Research Center West
Beltsville, Maryland 20705

Man Years:
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description:

Objectives: To determine if any relationship exists between maternal contact with blighted potatoes and the production of anencephaly of spina bifida.

Methods Employed: Animal feeding studies utilizing blighted potatoes and pregnant swine were performed.

Major Findings: Animal feeding experiments failed to demonstrate any ability of blighted potatoes to cause neural tube defects.

Significance of the Program to the Institute: The study failed to provide experimental support to Renwick's hypothesis that blighted potatoes are etiologically related to neural tube defects. The possibility that other environmental factors interacting with genetic influences should be intensively investigated.

Proposed Course of the Project: Terminated

Keyword Descriptors: Neural tube defects, blighted potatoes, animal feeding trials.

Honors and Awards: None

Publications: None

Serial No. Z01 NS 02087-02 IB
1. Infectious Diseases Branch
2. Section on Immunochemistry & Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Antenatal Diagnosis of Anencephaly and Spina Bifida by Maternal Serum Alpha Fetoprotein

Previous Serial Number: Same

Principal Investigators: Dr. Jerome E. Kurent, IDB, NINCDS
Dr. John L. Sever, IDB, NINCDS

Other Investigators: Mrs. Dorothy Edmonds, IDB, NINCDS
Mrs. Ruth Silvasco, IDB, NINCDS

Cooperating Units: Dr. Thomas Waldmann, NCI

Man Years:
Total: 0.3
Professional: 0.2
Other: 0.1

Project Description:

Objectives: To establish the value of maternal serum alpha fetoprotein (AFP) determinations in the antenatal diagnosis of anencephaly and spina bifida.

Methods Employed: Maternal serum AFP determinations of pregnancies resulting in anencephaly and spina bifida fetuses will be studied over the gestational period 12-20 weeks. Serum levels will be compared with pregnant women matched for gestational age and race. AFP determinations will be performed by the double antibody radioimmunoassay.

Major Findings: Results of a pilot study indicate that maternal serum obtained from anencephalic and spina bifida pregnancies have significantly elevated AFP concentrations in the majority of cases studied.

Significance of the Program to the Institute: If the clinical usefulness of maternal serum AFP determinations is established as a practical screening measure for the presence of neural tube defects, it will represent a valuable adjunct in the antenatal diagnosis of these severe congenital neurological defects.

Proposed Course of the Project: Data resulting from AFP determinations in neural tube defects are being evaluated. Additional malformation study groups will be studied for elevated AFP during pregnancy.

Keyword Descriptors: Anencephaly, spina bifida, alpha fetoprotein.

Honors and Awards: None

Publications: None

Serial No. Z01 NS 02070-02 ID
1. Infectious Diseases Branch
2. Section on Immunochemistry and Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Separation Techniques Involving Globulin Proteins in Cerebrospinal Fluid

Previous Serial Number: Same

Principal Investigators: Dr. Richard S. Johannes, IDB, NINCDS
Dr. Michael F. Murphy, IDB, NINCDS

Other Investigators: Otto Gutenson, IDB, NINCDS

Cooperating Units: None

Man Years:

Total:	.35
Professional:	.20
Other:	.55

Project Description:

Objectives: To develop separation techniques for proteins in cerebrospinal fluid and to use the techniques to examine particular families of proteins within CSF. This will allow for purification of measles specific antibody from CSF as well as examination of oligoclonal protein in cerebrospinal fluid.

Methods Employed: The main methodology employs the use of acrylamide gel electrophoretic techniques. Disc gel methods for banding at various pH ranges as well as iso-electric focusing will be used. Chromatographic techniques will also be used to obtain homogeneous protein fractions as starting points.

Major Findings: QAE sephadex columns will efficiently separate IgG from the remainder of protein in the CSF. However, there are serious questions as to the viability of the antibody binding sites after column passage for some antibodies. Techniques rising acrylamide gel are working well as are the iso-electric focusing techniques and promise a method for separation of subclasses of immunoglobulins in CSF.

Significance of the Program to the Institute: As significant attention has been paid to measles antibody and oligoclonal proteins in multiple sclerosis it seems logical to obtain these proteins in pure form. It is hoped that assays for these proteins and the antigens eliciting the oligoclonal IgG will shed light on possible etiologic factors in multiple sclerosis.

Proposed Course of the Project: To continue with improvements in the resolvi capacity of the iso-electric focusing and polyacrylamide electrophoretic techniques. The one point is obtaining pure fractions of oligoclonal immunoglobulins from CSF.

Keyword Descriptors: acrylamide gel, iso-electric focusing, disc electrophoresis, chromatography, immunoglobulin G

Honors and Awards: None

Publications: None

Serial No. Z01 NS 02071-02 ID
1. Infectious Diseases Branch
2. Section on Immunochemistry and Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cerebrospinal Fluid Electrophoresis-For Diagnosis and Prognosis of MS and SSPE

Previous Serial Number: Same

Principal Investigators: Dr. Richard S. Johannes, IDB, NINCDS

Other Investigators: None

Cooperating Units: University of Vermont (Dr. George Schumacher)

Man Years:

Total:	.75
Professional:	.75
Other:	0

Project Description:

Objectives: To examine isoenzymes of creative phosphokinase and lactate dehydrogenase in cerebrospinal fluid. These offer significant potential for indices of central nervous system cell damage in diseases such as MS, SSPE, and strokes. Fresh cerebrospinal fluids will be examined as made available. The globulin fraction will be examined with particular attention to a group of possibly homogeneous globulins accounting for the **oligoclonal** aspect. It is hoped that these proteins can be isolated and obtained in pure form.

Methods Employed: Free zone electrophoresis will be used to examine the isoenzymes of creatine phosphokinase and lactate dehydrogenase. These will be preconcentrated by ultrafiltration. Radioimmunodifusion will be used in order to determine IgG, IgA, and IgM levels in cerebrospinal fluid.

Major Findings: Eighteen patients with multiple sclerosis as well as several patients with other neurologic illnesses have been examined. Analysis is now in progress as to the usefulness of the isoenzyme levels as markers of clinical disease.

Significance of the Program to the Institute: The importance of demonstrating the diagnostic and/or prognostic significance to enzyme levels in cerebrospinal fluid lies in the fact that these tests are minor modifications of assays already present in many hospitals. Should they be found to have clinical meaning their utilization could be readily effected. The value of obtaining pure forms of the globulin fraction of CSF is that a family of proteins in this region has a close correlation with the clinical states of multiple sclerosis, and subacute sclerosing panencephalitis. Whether they represent primary or secondary pathological effects can not be known until they are obtained in pure form.

Proposed Course of the Project: To continue to examine varied techniques of purifying and subseparating proteins which migrate in the gamma globulin region. It is hoped that such efforts will further understanding of the so-called oligoclonal aspect. Concurrently we will examine isoenzymes of CPK and LDH in cerebrospinal fluid in hope that these biochemical tests in conjunction with close inspection of the gamma globulins will result in useful diagnostic tests for multiple sclerosis and subacute sclerosing panencephalitis.

Keyword Descriptors: isoenzymes

Honors and Awards: None

Publications: None

Serial No. Z01 NS 02130-01 ID
1. Infectious Diseases Branch
2. Section on Immunochemistry and Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Induction of Cellular Fusion by the Use of Cell Free
Systems and Its Relation to Virus Rescue

Previous Serial Number: None

Principal Investigators: Dr. Richard S. Johannes, IDB, NINCDS

Other Investigators: Otto Gutenson

Cooperating Units: None

Man Years:

Total: .25
Professional: .15
Other: .10

Project Description:

Objectives: To investigate the events leading to heterokaryon production and rescue of virus from chronic non-productive cell lines. The principal question is to ascertain whether living cells or a cell free extract of an indicator cell line is sufficient for the production of cellular fusion.

Methods Employed: The methods involve comparing the amount of fusion, cytopathic effect and viral liberation occurring during co-culture of chronic lines containing SSPE virus and indicator cell lines with mixing the chronic line and cell free extracts of the indicator cell line.

Major Findings: The addition of the membrane pellet of Vero cells but not the soluble fraction will cause significant cell fusion, similar to co-culture with living cells. However, the amount of virus released as infectious particles remains unanswered.

Significance of the Program to the Institute: If a cellular component which would cause the release of virus from a chronically infected cell line could be found, then investigation into levels of the same component in chronic lines could be assayed and perhaps an important control mechanism for non-productive viral infection of human tissues could be elucidated.

Proposed Course of the Project: To establish that a factor from Vero cells will cause cell fusion and/or release of virus. Secondly using isopicnic centrifugation to isolate the cellular fraction which actually leads to the effect, e.g. nucleii, mitochondria, microsomes etc. Once established these same studies could be run on chronic cell lines looking for differences.

Keyword Descriptors: chronic non-productive infection, productive infection, cell fusion, heterokaryon, co-culture, isopicnic centrifugation

Honors and Awards: None

Publications: None

Serial No. Z01 NS 02131-01 ID
1. Infectious Diseases Branch
2. Section on Immunochemistry and Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Determination of Antigenicity of DNA Viral Proteins for Vaccine Use

Previous Serial Number: None

Principal Investigators: Dr. Michael F. Murphy, IDB, NINCDS
Dr. David A. Fuccillo, IDB, NINCDS

Other Investigators: Otto Gutenson, IDB, NINCDS

Man Years:

Total:	.45
Professional:	.2
Other:	.25

Project Description:

Objectives: To obtain viral proteins which are nucleic acid free from DNA viruses, particularly those thought to be associated with neurological diseases. To develop an animal test system to evaluate the antigenicity of these proteins for possible vaccine usefulness.

Methods Employed: Proteins of viral origin obtained from infection of tissue cultures will be extracted by several means, e.g. sonication, treatment with detergents, and osmotic disruption. Purification of the viral proteins will be done with classical enzymological methods, in addition to the purification by isoelectric focusing. The latter methodology will achieve the required purification without loss of biological function. These proteins will be injected into model animal systems for evaluation of antigenicity.

Major Findings: At present there are no major findings available. The study is in the beginning stages.

Significance of the Program to the Institute: This study represents an initial effort into the field of prevention of possible neurological diseases, as opposed to treatment after the fact. It is hoped that information gained in these antigenicity studies will be correlated to other diseases being studied by this institute.

Proposed Course of the Project: Investigations of other DNA viruses associated with neurological diseases in primates can be extended from this study. Identification of the most antigenic viral protein and its associated function will hopefully give us a better understanding of how these diseases progress.

Keyword Descriptors: Antigenicity, DNA viruses, proteins, vaccine.

Honors and Awards: None

Publications: None

Serial No. Z01 NS 02132-01 ID
1. Infectious Diseases Branch
2. Section on Immunochemistry and Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Characterization of SSPE Viral-Like Particles in Latent and Lytic Tissue Culture Conditions.

Previous Serial Number: None

Principal Investigators: Dr. Michael F. Murphy, IDB, NINCDS
Otto Gutenson, IDB, NINCDS

Other Investigators: None

Man Years:
Total: .45
Professional: .20
Other: .25

Project Description:

Objectives: To study and identify changes in structural proteins and other biochemical differences occurring in the transformation of a lytic viral infection to the latent or chronic state where a viral genome or subunit resides inside a cell and causes no observable cytotoxicity. To ascertain the mode of replication of these two different states of infection.

Methods Employed: Nucleic acids which have been radio-labeled will be extracted from both types of infections and compared. The sizes of these genomes of particles will be obtained through gradient centrifugation and agarose chromatogrphy. Homology between species will be examined by nucleic acid hybridization. Viral proteins will be separated and sized by polyacrylamide electrophoresis and electrofocusing. Determination of protein functions will also be studied, e.g. RNA dependent RNA polymerase.

Major Findings: Preliminary findings indicate morphologically different particles associated with chronic infections as opposed to "normal" lytic infections. Actinomycin appears to inhibit both cellular synthesis and viral synthesis of nucleic acids. This may indicate a certain amount of cellular dependence for metabolic enzymes and/or functions. Electrophoretic patterns of proteins are now under investigation.

Significance to the Program of the Institute: This study will give information into the complexities of chronic or latent infections of which neurological diseases are thought to be a part. Identification of differences between lytic and chronic infection may be applied in the search for other agents causing neurological disease.

Proposed Course of the Project: Many of the lytic viruses appear to propagate defective particles which interfere with replication of the infective genome. Many viruses associated with diseases have been shown to be capable of entering a latent state in the host cell. It is proposed that this initial investigation be extended to include studies on CMV, Herpes, and other viruses in this category.

Keyword Descriptors: RNA, Proteins, SSPE chronic infection, PAGE, Nucleic acid hybridization.

Honors and Awards: None

Publications: None

Serial No. Z01 NS 02133-01 ID
1. Infectious Diseases Branch
2. Section on Immunochemistry and Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Enzymology as a Diagnostic Probe for Herpes and other DNA Viruses.

Previous Serial Number: None

Principal Investigators: Dr. Michael F. Murphy, IDB, NINCDS
Dr. David A. Fuccillo, IDB, NINCDS

Other Investigators: Otto Gutenson, IDB, NINCDS

Man Years:
Total: .45
Professional: .2
Other: .25

Project Description:

Objectives: To develop an assay of thymidine kinase activity to detect the possible presence of Herpes virus in certain disesases. To examine the possibility to extend these studies to other DNA viruses.

Methods Employed: Cytoplasm of control cells and chronically infected cells thought to contain Herpes viral genome are the source for the enzyme. An assay method for the enzyme is now being investigated. Polyacrylamide gel electrophoresis will be used to separate the different activities which have been shown to have different electrophoretic mobilities. This method will then be applied to examine CSF and serum of patients with neurological disease.

Major Findings: Under present conditions of assay, the thymidine kinase activity is very labile. Investigations are now under way comparing different methods of cell disruption and enzyme stabilization. For instance, it has been found that nonionic detergents have detrimental effects on this enzyme but do not affect RNA dependent RNA polymerase. Simple freezing and thawing of the cells appears to be the least harmful methodology in the preparation of this enzyme.

Significance of the Program to the Institute: This study is directed toward early identification of neurological diseases associated with viral antigens. Further, detection of antigens of DNA viruses known to be associated with some CNS diseases would alert the medical practioner to the possibility that babies of infected mothers should be closely examined and followed.

Proposed Course of the Project: To investigate the presence of either the entire genome or partial fragments of viruses which have been thought to be associated with neurological diseases, a detection assay of the specifically induced viral thymidine kinase activity will be used. The results of the assay system will be correlated to other diagnostic criteria such as virus isolation and fluorescent antibody tests.

Keyword Descriptors: Thymidine Kinase, polyacrylamide electrophoresis, Herpes virus, CSF.

Honors and Awards: None

Publications: None

Serial No. Z01 NS 02134-01 ID
1. Infectious Diseases Branch
2. Section on Immunochemistry and Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biochemical Effects of Antiviral Agents on Infected Cells and Control Cells.

Previous Serial Number: None

Principal Investigators: Dr. Michael F. Murphy, IDB, NINCDS

Other Investigators: Rebecca Hamilton, IDB, NINCDS

Man Years:

Total:	.4
Professional:	.2
Other:	.2

Project Description:

Objectives: To examine the biochemical effects exhibited by the antiviral, ribavirin, against control and infected cells in tissue culture. To compare and evaluate this synthetic nucleotide as an antiviral agent to be used against slow viruses containing either RNA or DNA.

Methods Employed: Tissue culture of control cells and cells infected with SSPE (Japanese) were exposed to ribavirin to determine the prolonged and initial effects on DNA synthesis, RNA synthesis, and protein synthesis. The reduction of infectious virions and plaque forming units will be examined and compared to varying concentrations of the antiviral. The drug will also be examined in whole animals to determine the detrimental effects (if any) on normal vs. infected animals.

Major Findings: Deoxyribonucleic acid synthesis and RNA synthesis are inhibited 90% within two hours of exposure to ribavirin in control vero cells. Protein synthesis seems not to be affected by the antiviral in our testing system. Infected cells (SSPE Japanese) display a different pattern of DNA and RNA synthesis when treated with ribavirin. The effect of inhibition appears not to be as severe as in control cases. Plaques are inhibited 90% in the presence of this drug. Animal studies and concentration are now being started.

Significance of the Program to the Institute: Any broad spectrum antiviral which is not toxic to the patient at effective dosage is important to any program. Indeed this antiviral may be tailored to neurological diseases in view of the differing results obtained between infected and non-infected cells.

Proposed Course of the Project: To investigate the effects of different levels of the antiviral and their efficacy. Also the examination of other viral agents, such as, DNA virus will be examined and compared to SSPE.

Keyword Descriptors: DNA synthesis, RNA synthesis, ribavirin, SSPE infected cells, Vero cells.

Honors and Awards: None

Publications: None

Serial No. Z01 NS 02135-01 ID
1. Infectious Diseases Branch
2. Section on Immunochemistry and Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Rapid Diagnosis of Meningitis

Previous Serial Number: None

Principal Investigators: Dr. John L. Sever, IDB, NINCDS

Other Investigators: Dr. David A. Fuccillo, IDB, NINCDS

Cooperating Units: None

Man Years:

Total:	.50
Professional:	.25
Other:	.25

Project Description:

Objectives: To develop more rapid methods for the diagnosis of meningitis.

Methods Employed: New micro limulus tests are being studied for the detection of endotoxin in spinal fluid. New counter immunoelectrophoresis methods and latex methods are being tried for specific identification of bacterial antigens.

Major Findings: Limulus lysate, properly prepared is a highly sensitive and accurate method for detection of gram negative organisms in CSF. Immunoelectrophoresis appears to identify major bacterial antigens in the CSF.

Significance of the Program to the Institute: The rapid diagnosis of meningitis is extremely important for the prevention of serious brain damage to death. These methods appear to be of considerable value.

Proposed Course of the Project: A total of 300 spinal fluid specimens will be tested with the limulus, counter immunoelectrophoresis and latex methods to determine sensitivity and reliability. New method will be added.

Keyword Descriptors: Acute infections, diagnosis, limulus, counter immunoelectrophoresis

Honors and Awards: None

Publications: None

Serial No. Z01 NS 01731-07 ID
1. Infectious Diseases Branch
2. Section on Virology and Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Isolation of Infective Agents From Chronic Diseases

Previous Serial Number: Same

Principal Investigators: Dr. Luiz H. Barbosa, IDB, NINCDS
Dr. David A. Fuccillo, IDB, NINCDS
Dr. William T. London, IDB, NINCDS
Dr. John L. Sever, IDB, NINCDS
Dr. Monique Dubois-Dalcq, IDB, NINCDS
Dr. Jerome Kurent, IDB, NINCDS

Other Investigators: Mrs. Rebecca Hamilton, IDB, NINCDS
Mr. Otto Gutenson, IDB, NINCDS
Mrs. Anita C. Ley, IDB, NINCDS
Mr. Leonard Moore, IDB, NINCDS

Cooperating Units: University of Tennessee (Dr. J. T. Jabbour)
University of Vermont (Dr. George Schumacher)
Indiana University Medical Center (Dr. Wolfgang Zeman)
Wilmington General Hospital (Dr. George Boines)
Cornell University, N. Y. (Dr. Fred Plum)

Man Years:

Total:	4.0
Professional:	1.5
Other:	2.5

Project Description:

Objectives: To establish whether persistent or tolerant viral infections are associated with chronic diseases such as Bell's palsy, amyotropic lateral sclerosis, Creutzfeldt-Jakob disease, progressive multifocal leukoencephalopathy, and multiple sclerosis.

To purify and concentrate PML and SSPE viruses and examine these agents electron microscopically, biochemically, and antigenically in parallel with the conventional forms of these viruses.

To attempt the transmission of MS to SPE mice and chimpanzees using intracerebral inoculation of 10% homogenate prepared with "plaque" areas from brain autopsy material.

To trace the possible anti brain activity of MS immunoglobulin, using I^{131} labeled MS serum to inoculate Rhesus monkeys for brain autoradiography. To develop an animal model system to study the immunopathogenesis of SSPE as well as possible therapeutic approaches.

To conduct epidemiological surveys concerning the occurrence of SSPE during the 1965-1975 decade.

To evaluate the role of cellular immunity, humoral immunity, and interferon in protecting experimental animals against neurotropic measles virus.

Methods Employed: Brain specimens from well documented cases of MS, PML, ALS, SSPE, etc., were homogenized to a 10% suspension and inoculated intracranially in groups of 3 Rhesus monkey fetuses during the first one third of gestation when the animals are expected to be immunologically immature and hence more susceptible to infectious agents. These fetuses were carried to term and, immediately after birth, one animal was killed and examined histopathologically for CNS lesions. The remaining animals were carefully observed for clinical symptoms, abnormal behavior, and antibody pattern. A 3 year follow up has been scheduled.

Brain tissue from patients with encephalopathies, muscle from patients with polymyositis, and lymph nodes from individuals with SSPE and MS were examined by electron microscopy, fluorescent microscopy and, whenever possible, cultured in vitro. These tissue cultures were submitted to virologic and serologic assays, inoculated into experimental animals, and co-cultivated with human diploid and heteroploid cell lines in efforts to isolate and identify intracellular agents.

Comparative studies between SSPE measles and prototype measles virus were conducted in efforts to establish whether or not the SSPE isolates have markers to distinguish them from laboratory strains of measles.

A questionnaire concerning the occurrence of SSPE since 1956 was sent to neurologists, pediatricians and pediatric neurologists at 105 medical centers throughout the USA. Data was analyzed in our laboratory and at the University of Tennessee.

In order to investigate a possible impairment of cellular immunity in SSPE patients, an in vitro lymphocyte assay was conceived and efforts initiated to obtain a measles chronically infected cell line necessary to evaluate lymphocyte measles-specific delayed hypersensitivity.

Major Findings: Primary and secondary MS brain cultures were extensively tested for the possible presence of viruses. No evidence of an infectious agent was found in these MS brain cultures studied by immunofluorescence, electron microscopy, hemadsorption, co-cultivation, and biochemical "shocking".

An immunoperoxidase electron microscopic test for measles virus was developed and used to study SSPE strains of the virus. This assay is expected to be useful in the search for antigens in MS, PML, and other neurological diseases.

The search for biological markers in distinguishing SSPE isolated from conventional measles virus proved successful. Marked differences between SSPE strains were noted by various parameters, indicating strain dissimilarities as great or even greater than those previously observed between laboratory strains of measles virus.

Epidemiological studies involving over 250 SSPE patients and dozens of medical institutions produced the following major findings: 1) the frequency of SSPE was approximately 1:1,000,000 during 1960-1970, 2) concentration of patients in the southeastern part of the U.S., 3) the age of onset of the disease was 7.2 years, 4) a 3:1 male/female ratio, and 5) early measles infection < 3 years of age in the majority of patients.

Significance of the Program to the Institute: The development of mixed cell cultures to unmask latent infections provides an excellent methodology for the study of chronic diseases of possible viral etiologies.

The understanding of slow virus infections of the CNS will depend upon purification of the suppressed form of these agents followed by careful biochemical and biophysical analysis. To accomplish this goal, the use of zonal centrifugation for cellular fractionation should be emphasized.

Proposed Course of the Project: Special emphasis will be placed upon a brain culture research program. Investigation of the mechanism of pathogenesis and possible immune deficiencies in patients with neurological diseases will be conducted. Our selection of patients with multiple sclerosis, Parkinson's disease, progressive multifocal leukoencephalopathy, and amyotrophic lateral sclerosis is supported by the existing data which suggest possible viral etiologies for each of these diseases. Tissue specimens and blood from patients will be provided through collaborative contract arrangements with investigators throughout the country.

The possible viral etiology for MS will be exhaustively investigated. Since the pathology of Visna and MS are similar, and reverse transcriptase was found in tissues infected with Visna virus, we intend to determine whether this viral enzyme is present in spinal fluid and brain tissue of MS patients. In addition, molecular hybridization studies with MS tissue and tissue cultures will be initiated to search for viral "fingerprints" such as messenger RNA, virus specific DNA or RNA. These homology assays would be conducted with MS purified DNA and RNA in presence of labelled measles RNA and possibly labelled varicella-zoster DNA. The latter viruses are at present the best candidates since serologic surveys indicated that MS patients have elevated measles and/or zoster antibody levels.

In a second approach, MS gamma globulins will be coupled to tracers (fluorescein and peroxidase) and used in a direct antigen-antibody test with MS brain tissue cultures. Using the electron microscope and UV microscope, we can determine and identify antigenic sites in the MS brain tissue culture

Third, in a more direct fashion, we will utilize experimental animals known to be surgically acceptable to slow neurotropic agents (scrapie, visna) to inoculate MS brain preparations. These animals will include SPF mice and primates.

Utilizing the mixed culture technique, we hope to determine whether it is possible to release suppressed virus from these chronic neurologic diseases and to gain a further understanding of the pathogenesis of latent infections of the CNS. Antibody levels and competence of lymphocytes from patients will be examined, using the standard techniques. The SSPE strain of measles virus will be studied in laboratory animals and efforts will be directed at the development of an animal model system for this disease.

Keyword Descriptors: Multiple Sclerosis, Amyotrophic Lateral Sclerosis, Progressive Multifocal Leukoencephalitis, Subacute Sclerosing Panencephalitis, Creutzfeldt-Jakob Disease, purification and identification of agents, transmission experiments, immunopathogenesis.

Honors and Awards: None

Publications:

Dubois-Dalcq, M., Barbosa, L. H., Hamilton, R., and Sever, J. L.: Comparison Productive and Latent Subacute Sclerosing Panencephalitis Infection in Vitro. Lab. Invest. 30: 291-299, 1974.

Raine, C. S., Feldman, L. A., Sheppard, R. D., Barbosa, L. H., and Bornstein, M. B.: Subacute Sclerosing Panencephalitis Virus: Observations on a Neuroadapted and Non-neuroadapted Strain in Organotypic CNS Cultures. Lab. Invest. 31: 42-53, 1974.

Dubois-Dalcq, M., Worthington, K., Gutenson, O., and Barbosa, L. H.: Immunoperoxidase Labeling of Subacute Sclerosing Panencephalitis (SSPE) Virus in Hamster Acute Encephalitis. Lab. Invest. (In Press).

[illegible]

[illegible]

Project Title: [illegible]

[illegible]

Principal Investigators: [illegible]

Other Investigators: [illegible]

Cooperating Site: [illegible]

Man Years:

Total: [illegible]
Professional: [illegible]
Other: [illegible]

Program Description

Objectives: [illegible]

[illegible]

[illegible]

Major Findings: The study on occurrence of mycoplasma in males has been continued. Classical strains mycoplasma were recovered from about 10% of the males studied in the vasectomy clinic, at the National Naval Medical Center, and T-strain mycoplasma were isolated from about 20%. The isolations were made from both urine and semen but not from vasectomy tissue indicating that semen was contaminated during the passage through the urethra. Previous studies on pregnant women in the same facility demonstrated that they had a similar frequency of mycoplasma in the reproductive tract. Mycoplasma in the pregnant women could not be associated with birth of abnormal children, although the newborn of these carrier women were often contaminated with mycoplasma. In an effort to find a population with a higher frequency of mycoplasma, 50 marines with nonbacterial urethritis and 50 normal marines at the Quantico marine base, in Virginia were cultured. The frequency of mycoplasma in this population seems to be somewhat greater than the Navy population. However, the frequency of mycoplasma in men with nonbacterial urethritis did not seem to be signficantly higher than in the control marines without urinary tract infections.

Studies of tissue culture lines, seed viruses used to produce antigens or antibodies and primary isolation tissue indicate that mycoplasma often occur as contaminants when least expected. Continual monitoring of common reagents used in infectious disease studies is necessary to prevent these contaminating agents from producing interfering results.

Significance of the Program to the Institute: A program devoted to studying the effects of mycoplasma in various diseases complements the virological studies currently being done. This study and its support given to other investigators may help to more accurately define the role of mycoplasma and other agents in disease.

Proposed Course of the Project: Efforts are underway to initiate a study to determine the frequency of mycoplasma in amniotic fluid and in infertility and repeat abortion. Attempts to define the role of mycoplasma in neurological diseases will be continued. Continued efforts will be made to apply new techniques in the identification of mycoplasma and mycoplasmal disease.

Keyword Descriptors: mycoplasma, abortion, urinary tract infection, tissue contamination mycoplasma

Honors and Awards: None

Publications:

Madden, D. L., Moats, K. E., London, W. T., Matthew, E. B. and Sever, J. L.: Mycoplasma Moatsii, a New Species Isolated from Recently Imported Grivit Monkeys (Cercopithecus aethiops). International Journal of Systematic Bacteriology, Vol. 24, No. 4, pp. 459-464, October, 1974.

with Electro-nucleonics 12 samples of serum and feces have been examined by electron microscopic and immunoelectron microscopic techniques for presence of hepatitis A antigen. Serum from mothers of abnormal children and controls were obtained from the perinatal study and tested for presence of hepatitis B antigen by radioimmunoprecipitation techniques.

Major Findings: Epidemiological studies in institutionalized mentally retarded patients have provided additional information about the cause of death in these patients. In this study, patients with Down's Syndrome (DS) were matched with other mentally retarded patients (OMR) as to age, sex, resident ward, age at first institutionalization. Our data indicates that over the past five years the death rate in patients with (DS) is approximately twice that of OMR patients. The death rate among patients with chronic hepatitis B antigenemia is about twice that of patients without hepatitis B antigenemia. The death rate of Down's Syndrome patients with hepatitis B antigenemia is 4 times that of all other patients.

Epidemiological studies on hepatitis A in institutionalized patients have been continued. Typical hepatitis A particles have been demonstrated in 4 of 12 fecal samples studied. These samples were collected at the time of peak SGPT rise. Serological studies (immunoelectron microscopic techniques) on patients serum who did not develop hepatitis A in the outbreak indicated that they had circulating antibodies prior to the epidemic.

Studies on the association of hepatitis in pregnant women and observable disease in newborns has been continued. Preliminary studies indicate that the etiology of Down's Syndrome is not associated with hepatitis A. In 700 serum samples obtained from the perinatal study, hepatitis B antigen was not detected in the serum of mothers with abnormal children at a higher incidence then mothers with normal children.

Significance of the Program to the Institute: Hepatitis in pregnancy has been associated with neonatal jaundice, Down's Syndrome and other forms of mental retardation. Among the institutionalized mentally retarded patients those with trisomy 21 karyotypes have a higher rate of chronic hepatitis B antigenemia than others. This study provides additional depth to determining the role of infectious agents in perinatal disease and mental retardation. Data obtained from this study will clarify the association of hepatitis with various perinatal diseases and mental retardation.

Proposed Course of the Project: Studies on the effect of hepatitis B in a mentally retarded population are being completed. The relationship between exposure to hepatitis B antigen and neonatal jaundice, hepatitis and mental retardation are being determined. Efforts are being made to initiate studies which will determine if any etiological relationship between neonatal jaundice and mental retardation and hepatitis A infection exists.

Keyword Descriptors: mental retardation, Hepatitis B, Hepatitis A, Down's Syndrome, and perinatal infections.

Awards and Honors: None

Publications:

Madden, D. L., Dietzman, D. E., Matthew, E. B., Fuccillo, D. A., Hall, W. T., and Bond, H. E. and Rostafinski, M.: Distribution of Serotypes of Hepatitis B Surface Antigen in an Institution for the Mentally Retarded. Journal of Infectious Diseases, 131: 439-442, 1975.

Serial No. Z01 NS 01987-04 ID
1. Infectious Diseases Branch
2. Section on Virology and Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunoglobulin M and Congenital Infections

Previous Serial Number: Same

Principal Investigators: Dr. Luiz H. Barbosa, IDB, NINCDS
Dr. John L. Sever, IDB, NINCDS

Other Investigators: Miss Helen Krebs, IDB, NINCDS
Mrs. Rebecca Hamilton, IDB, NINCDS
Mr. Otto Gutenson, IDB, NINCDS

Cooperating Units: University of Tennessee (Dr. Sheldon Korones)
Electro-Nucleonics, Inc. (Dr. Edward Bond)

Man Years:

Total:	0.8
Professional:	0.2
Other:	0.6

Project Description:

Objectives: To study the antibody specificity of cord and newborn serum IgM as an investigational screening procedure to identify intrauterine or neonatal infections. High risk pregnancies will be included in this study, using the elevated IgM level as index for possible perinatal infections.

Methods Employed: Part of this investigation is being studied in collaboration with Dr. Korones at the University of Tennessee. In these studies, serum specimens are obtained bi-weekly from newborn infants in the high risk nursery. Elevated IgM levels (> 20 mg. %) will be used as indication of possible congenital or neonatal infection. Sera with high IgM content are then passed through prepared sepharose columns in order to separate the 7S from the 19S gamma globulins. Purified IgM samples are subsequently tested by the indirect immunofluorescent assay against CMV, HVH, Rubella, Toxoplasma, and Treponema Pallidum which are known to be the main causative agents of perinatal infections. Infected tissue cultures are used as the subs rate assay for the three viruses and toxoplasma and treponema smears are utilized for determining IgM antibody elicited by toxoplasmosis and syphilis, respectively. A fluorescein conjugated anti-IgM serum is employed to stain antigen-antibody complexes. A careful longitudinal follow-up of the children under study will be conducted by Dr. Korones to correlate serological data with clinical observations, and when indicated, clinical specimens will be sent to the laboratories of the Infectious Diseases Branch.

A second part of this project involves the separation and antibody determination of the IgM in approximately 12,000 cord serum samples from babies with elevated IgM. These infants' samples are from the Collaborative Perinatal Study and are products of conception from mothers with serological findings indicating infections during pregnancy. Antibody specificity of the purified cord serum IgM samples are tested in the same manner described above.

Major Findings: The novel technique of antibody-coupled sepharose particles was perfected in collaboration with Dr. Bond (Electro-Nucleonics) and found to be useful to separate immunoglobulins from body fluids. This technique provides an inexpensive and rapid method to purify IgM in contrast with the elaborate, time-consuming preparative ultracentrifugation in sucrose gradients.

Measles specific IgM was found to be present in the serum of SSPE patients. This observation correlates with an early report by Connoly, et al., describing rubeola antibody in the IgM fraction of SSPE patient's spinal fluid.

Significance of the Program to the Institute: Several infectious agents are known to produce intrauterine infection and fetal damage. A great number of mentally retarded children are a consequence of congenital infection affecting the CNS. Cord and newborn serum IgM levels are usually elevated with congenital infections. Therefore, the quantitation and qualitation of cord and newborn serum IgM can be applied as an investigational screening procedure to identify high risk infants, or in studies of newborn in distress. More specific procedures can then be used to determine the cause for the elevation of this immunoglobulin.

Proposed Course of the Project: Comparison studies between specific immunofluorescence and latex agglutination techniques will be conducted to evaluate degree of sensitivity and specificity of cord IgM antibodies and candidate antigens (Toxoplasma, syphilis, CMV, rubella, and Herpes). Once the assay is perfected to a desirable reproducibility, approximately 1,800 high IgM and serum samples from the Collaborative Perinatal Research Study will be tested for specific IgM antibodies. The results will be correlated with the mother's serum antibody findings as well as mother's clinical history and the infant's findings. Due to technical difficulties this project was discontinued.

Keyword Descriptors: intrauterine infections, cord serum, immunoglobulin M, antibody specificity, immunofluorescent assays.

Honors and Awards: None

Publications: None

Serial No. Z01 NS 01982-04 ID
1. Infectious Diseases Branch
2. Section on Virology and Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Delayed Hypersensitivity in Chronic Viral Diseases

Previous Serial Number: Same

Principal Investigator: Dr. David A. Fuccillo, IDB, NINCDS

Other Investigators: Dr. David Madden, IDB, NINCDS
Mrs. Renee Traub, IDB, NINCDS

Cooperating Units: Dr. Joseph Bellanti, Georgetown University
School of Medicine, Washington, D.C.
Dr. Russell Steele, Brooke Army Medical Center
Fort Sam Houston, Texas
Mr. Monty Vincent
Microbiological Associates, Bethesda, Md.

Man Years:

Total:	1
Professional:	0.5
Other:	0.5

Project Description:

Objectives: To determine the role of delayed hypersensitivity (cellular immunity) in chronic viral infections.

Methods Employed: A radioactive aliquot transfer technique for cell mediated immunity was developed which will increase the number of specimens that can be done at one time.

A lymphotoxin assay and a antibody complement mediated test for rubella and rubeola have been developed. Other tests for CMV and Herpes are being worked on at the present time.

A rubella chronically infected cell line was found to be very useful for the lymphotoxin assay. This assay was developed and tested against immunized rubella children and results indicate a workable system is now available.

A rubeola chronically infected cell line was developed. This system is now being used in the study of SSPE and MS patients to determine their cellular immune capabilities against this virus.

A leukocyte inhibitory factor test (LIF) was established in our laboratory to study the capabilities of MS patients and controls against a number of viral antigens.

Major Findings: Specific cell-mediated immunity to rubella virus was studied in children immunized with rubella and employing a ^{51}Cr microassay. Results suggest that rubella immunization produces cellular immune response as early as 4-7 days after inoculation whereas immune response takes 10-14 days before being detectable. The cellular immunity of MS patients, using the LIF test, was found to be lacking in a specific recognition for measles antigen. A lymphocytotoxic test using a rubeola target cell demonstrated the presence of an inhibitor in the spinal fluids of some SSPE patients.

Significance of the Program to the Institute: Prior to the development of the above tests, no reliable tests for measuring cellular immunity in humans to viral antigens existed. Antibody tests to viral antigens have been developed and used in this and other laboratories for many years. This represents a test for only part of the body's immune response. The development of lymphotoxin and the LIF assays into a clinically useable test for virus infection now allows both forms of the body's protective immune mechanism, humoral and cellular, to be tested simultaneously. Treatment of patients found to have any defects may then be possible.

Proposed Course of the Project: To complete the above mentioned specific projects and to develop additional tests to elucidate how the cellular immune system is involved with other viral diseases, especially those that cause congenital infection. Congenitally infected infants excrete virus in the presence of high antibody titers, possibly indicating that their cellular immune system is impaired. The effect of complement as well as the function of cells other than lymphocytes can be studied with this system. These tests will also be used to determine the cellular immune capabilities of patients with MS and other neurological diseases. These tests will also be used to detect cell surface changes of infected cells.

Keyword Descriptors: Cellular immunity, delayed hypersensitivity.

Honors and Awards: None

Publications: Steele, R.W., Hensen, S.A., Vincent, M.M., Fuccillo, D.A., and Bellanti, J.A.: The Development of Specific Cellular and Humoral Immune Responses in Children Immunized with Live Rubella Virus Vaccine. J. Infectious Diseases, May, 1975.

Fuccillo, D.A., Abela, Jane E., Traub, R.G., Gillespie, M.M., Beadle,E.L., Sever, J.L.: Cellular Immunity in Multiple Sclerosis. The Lancet, May 1975.

Serial No. Z01 NS 01983-04 ID
1. Infectious Diseases Branch
2. Section on Virology and Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Chronic Viral Infections

Previous Serial Number: Same

Principal Investigator: Dr. David A. Fuccillo

Other Investigators: Dr. John L. Sever, IDB, NINCDS
Dr. William T. London, IDB, NINCDS
Dr. David L. Madden, IDB, NINCDS
Mrs. Flora Moder, IDB, NINCDS
Mrs. Renee Traub, IDB, NINCDS

Cooperating Units: Dr. L. Johnson
Harvard Medical School, Boston, Massachusetts
Dr. Mark May
McMillian Hospital, St. Louis, Mo.
Dr. Gary L. Gitnick
Dept. of Medicine, UCLA Center for Health Sciences
Los Angeles, California
Mr. Monty Vincent, Microbiological Associates
Dr. McLean, Naval Medical Center, Bethesda, Maryland
Dr. John Klousia, Quantico Marine Base, Quantico, Va.

Man Years:		
	Total:	2.0
	Professional:	1.0
	Other:	1.0

Project Description:

Objectives: The main objective of this project is to establish the clinical and biological significance of the two different strains of herpes simplex virus (Type I, "oral" and Type II, "genital") cytomegalovirus, varicella, in the causation of human disease including carcinoma, ulcerative colitis, Bells' palsy, facial paralysis, herpes zoster and chronic neurological diseases.

Methods Employed: The principal methods employed are: (1) the indirect hemagglutination test, by which type specific herpesvirus antibody is identified; (2) mass serological surveys of materials from selected patients with special disease entities; (3) virus isolation, titration, and characterization procedures; (4) monolayer cultivation of tissue with cocultivation; (5) fluorescent antibody studies of monolayer cultures for the presence of latent infection.

Major Findings: A number of serial serum specimens were collected by Dr. Johnson on primary herpes infections. Comparison serology were done and indirect hemagglutination was found to be the most specific test for Herpes type II. In collaboration with Dr. Gitnick, a number of sera from ulcerative colitis patients were serologically studied. CMV titers were still higher with the ulcerative colitis patients. Acute and convalescent sera from Bells palsy patients were studied with CMV, Herpes and Varicella antigens. No significant changes in titer of antibodies could be detected. In collaboration with Dr. Klousia, approximately 150 chronic urethritis and controls were studied for mycoplasma, Herpes, CMV and Trachoma. Results are being summarized.

In collaboration with Dr. McLean, a preliminary study was done to determine if the male was a reservoir of infections for cytomagalovirus, herpes and mycoplasma. Results were reported. An additional 100 specimens including serum and urine were studied and results are being summarized.

Significance of the Program to the Institute: These investigations attempt to elucidate the pathogenesis of viral infections of the adult and fetus using immunological and virological techniques. Herpes simplex virus, CMV, and varicella have been agents which have received particular attention in these studies, since they have significant neurotropic capabilities in terms of newborn and adult encephalitides. There is considerable speculation that these viruses may have latent "slow" virus potential in relationship to chronic diseases of humans, including carcinoma and central nervous system infection. Investigation of the clinical and biological properties of the two strains of herpes simplex virus have permitted more definitive establishment of such capabilities. Furthermore, the therapeutic potential of experimental drugs in animals with these infections may prove useful for future treatment of humans.

Proposed Course of the Project: Antibody complement mediated cytotoxicity studies with serum from carcinoma in situ patients will be studied. A study of PML, SV_{40} in owl monkeys will be done to determine if PML can be produced in experimental animals. The carp agent will be examined for possible relationship to MS. An infectious mononuclear antigen will be produced for possible diagnostic test using indirect hemagglutination test.

Keyword Descriptors: Chronic infections, Herpes viruses.

Honors and Awards: None

Publications: Fuccillo, D.A., Kurent, J.E., and Sever, J. L.: Slow Virus Diseases. Annual Review of Microbiology 28: 231-264, 1974.

Oldstone, M.B., Bokisch, V.A., Dixon, F.J., Barbosa, L.H., Fuccillo, D.A., and Sever, J.L.: Subacute Sclerosing Panencephalitis: Destruction of Human Brain Cells by Antibody and Complement in an Autologous System, Clin. Immuno. Immunopath. (In Press).

Serial No. Z01 NS 01984-04 ID
1. Infectious Diseases Branch
2. Section on Virology and Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Maternal Infection and Pregnancy Outcome

Previous Serial Number: Same

Principal Investigators: Dr. David A. Fuccillo, IDB, NINCDS
Dr. John L. Sever, IDB, NINCDS

Other Investigators: Dr. William T. London, IDB, NINCDS
Mrs. Renee Traub, IDB, NINCDS
Mrs. Mary Ruth Gilkeson, IDB, NINCDS
Mrs. Flora Moder, IDB, NINCDS

Other Investigators: Dr. William T. London, IDB, NINCDS
Mrs. Renee Traub, IDB, NINCDS
Mrs. Mary Ruth Gilkeson, IDB, NINCDS
Mrs. Flora Moder, IDB, NINCDS

Cooperating Units: Pennsylvania Hospital (Dr. Corson and Dr. Bolognese)
Naval Medical Center, Bethesda, Md. (Dr. Billingsley)
University of Tennessee,(Dr. S. Korones)
University of Cincinnati, (Dr. G. Altschuler)

Man Years:
Total: 4.5
Professional: 1.0
Other: 3.5

Project Description:

Objectives: Of the 3.5 million children born each year in the U.S. approximately 3% are mentally retarded. It appears that as many as 10% of these cases may be attributed to infectious disease. Our objective is to utilize various virological techniques in an intensive study of viruses to determine their role in the production of birth defects and related abnormalities. To develop virological techniques necessary for the investigation of the natural course of the disease as caused by the infectious agents.

Methods Employed: New virus isolation techniques and serum neutralization tests are used for large scale testing in the study of pregnant women and their children. The development of new techniques, such as the IHA test for Herpes hominus Types I and II and a new test for cytomegalovirus will now permit the determination of the frequency of virus experience among study populations along with the presence of antibody and change in antibody titer to the virus. These tests are being used to establish the reliability of other tests and to determine their sensitivity and specificity. A serologic study utilizing these tests on sera collected on the Collaborative Study population was conducted to determine the frequency of antibody changes during pregnancy and the effect of these infections on the developing embryo. Babies born with high IgM are being cultured and serological studies done to determine if fetal infection occurred. Neonatal deaths and spontaneous abortions were studied to determine abnormal outcomes.

Rubella vaccine was given to pregnant women negative for rubella (at the Pennsylvania Hospital) who were subsequently being aborted. Study is completed and is being reported.

Pregnant women will be studied for genital infection with herpesvirus and cytomegalovirus. The effects of virus on pregnancy during gestational period will be studied. Amniotic fluid will be taken during pregnancy to determine presence of virus and IgM determinations performed on the fluids to ascertain the presence of this globulin and its value in the early detection of in utero infection. Follow up on patients with herpes will be done for abnormal Pap smears and cancer of the cervix.

Major Findings: Naval Hospital isolation rates for CMV from mothers cervixes and babies urine were found to be 1.2% and .2% respectively. Rates for herpes was .09% for mothers and .3% for babies. The Philadelphia viral isolation study on pregnant women were CMV .78% and Herpes .65%. No increased IgM were found in any of the amniotic fluids studied. A number of viral isolations were made from the high IgM study and are being summarized. A number of c type particles were seen in the placental tissue of neonatal death cases and the isolation of a virus from a patent ductus arteriosus. Isolation studies on tissues from patients of hysterotomies are completed and summarized. The fetal abortion studies, after rubella vaccinations, have been extended and results will be reported. Virus was isolated from products of conception in the patients.

Significance of the Program to the Institute: The results from these studies help determine what effect virus infection has on abnormal pregnancy outcomes and also provide valuable information on the epidemiological aspects of virus infections. The relationship of these in utero infections to various degrees of neurological dysfunction in later years of life is still unknown.

Proposed Course of the Project: Studies are still in progress on cytomegalovirus infections during pregnancy and in several population groups. Additional studies are being conducted on Herpes Type I and II infections in women. Drug evaluation of Phosphoacetic acid for CMV and Herpes hominis is being conducted in monkeys. Experimental drugs are also being tested. Approximately 16,000 sera from abnormal pregnancy outcomes are being tested for Herpes I and II and CMV.

Keyword Descriptors: Perinatal Infections, Teratology

Honors and Awards: None

Publications: Madden, D.L., Dietzman, D.E., Matthew, E.B., Fuccillo, D.A., Hall, W.T., Bond, H.E., and Rostafinski, M.: Distribution of Serotypes of Hepatitis B Surface Antigen in an Institution for the Mentally Regarded. Journal of Infectious Diseases, Vol. 131, No. 4, pp. 439-442,

Sergo, S., Reynolds, D., Fuccillo, D.A.: Cervical Cytomegalovirus Excretion in Pregnant and Nonpregnant Women: Suppression in Early Gestation. J. Infect. Dis. May 1975.

Serial No. Z01 NS 01985-04 ID
1. Infectious Diseases Branch
2. Section on Virology and Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Isolation and Identification of Viral Antigens and Antibody for Rapid Identification of Virus Strains and Diagnosis of Diseases

Previous Serial Number: Same

Principal Investigators: Dr. David L. Madden, IDB, NINCDS

Other Investigators:
Dr. David A. Fuccillo, IDB, NINCDS
Dr. Luiz H. Barbosa, IDB, NINCDS
Dr. Gabriel Castellano, Microbiological Associates, Inc.
Mrs. Mary A. Krasny
Mrs. Aurella Krezlewicz

Cooperating Units: Microbiological Associates, Inc.

Man Years:

Total	1.75
Professional:	.50
Other:	1.25

Project Description:

Objectives: To isolate and identify several viral antigens from each of several viruses. To utilize these antigens for more specific, rapid, sensitive identification of antibody, and/or a more accurate identification of the viral agent.

Methods Employed: Human tissue culture lines chronically infected with SSPE measles virus, herpes virus type I and herpes virus type II have been used to determine the immunological response of MS patients and matched pal controls to these antigens. The infected cells have been labeled with 51 chromium. Target cells and various concentrations of lymphocytes or serum are reacted for 18 and 24 hours and the amount of 51 chromium specifically released is determined.

Simian hemorrhagic fever (SHF) an acute viral disease of rhesus monkeys and a chronic viral disease in other simian species has been studied. Lack of an *in vitro* diagnostic test has hampered studies of this disease. A highly specific SHF antibody has been produced by inoculating infectious rhesus monkey serum into guinea pigs. These animals are bled and normal rhesus serum antibodies are absorbed using normal rhesus monkey serum fixed in an acrylamide matrix leaving the specific SHF antibody. A complement fixation test using the specific SHF guinea pig antibody and infectious rhesus monkey serum has been developed. Antibody against the tissue culture adapted strain has been produced.

Major Findings: A technique for the rapid transfer of radioactive material from microtiter plates to the counting chamber has been developed. These tips have eliminated much of the nonspecific release encountered with other techniques. In an initial study it appears that there is no significant difference between the humoral or cellular immunity of patients with MS and matched controls to measles virus.

A highly specific antibody against SHF has been produced in guinea pigs which reacts in a complement fixation with antigen in blood of acutely ill rhesus monkeys and chronically infected Patas and African Green monkeys. The tissue culture produced antigen does not react against this serum but one passage in the rhesus monkey resulted in the development of a reactive antigen. This indicates that the tissue culture adapted strain has lost some antigenic properties. Isolation of this antigenic property could result in the development of a vaccine which would aid in control of this disease.

Significance of the Program to the Institute: The development of more specific antigens or antibodies which measure more accurately the immunological status of an individual is needed. Highly specific antigens or antibody may help identify the biological differences between nonpathogenic and pathogenic strains of these organisms and identify the etiology of obscure diseases. The studies on SHF will aid in determining the epizootiology of the disease and aid in eliminating another disease in animals used for human research models.

Proposed Course of the Project: Further studies will be done to identify the antigens associated with the measles and rubella, Herpes I, Herpes II, and CMV, and SHF viruses. Cellular and humoral immunity studies are being expanded in an effort to detect small amounts of antigen on intact cells and immunological response differences which may account for disease. Efforts will be made to utilize the immune electron microscopic technique in detection of agents associated with multiple sclerosis and other neurological diseases of unknown etiology.

Keyword Descriptors: multiple sclerosis, cellular immunizing, simian hemorrhagic fever, herpes virus, measles virus.

Honors and Awards: None

Publications: None

Serial No. Z01 NS 01991-04 ID
1. Infectious Diseases Branch
2. Section on Virology and Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunologic Studies of Congenital Infections and Chronic Infections

Previous Serial Number: Same

Principal Investigators:
Dr. Luiz H. Barbosa, IDB, NINCDS
Dr. David A. Fuccillo, IDB, NINCDS
Dr. William T. London, IDB, NINCDS
Dr. John L. Sever, IDB, NINCDS
Dr. Jerome Kurent, IDB, NINCDS
Dr. Monique Dubois-Dalcq, IDB, NINCDS

Other Investigators:
Mrs. Rebecca Hamilton, IDB, NINCDS
Mr. Otto Gutenson, IDB, NINCDS
Miss Helen Krebs, IDB, NINCDS
Mr. Leonard Moore, IDB, NINCDS
Mrs. Anita C. Ley, IDB, NINCDS

Cooperating Units:
Wilmington General Hospital (Dr. George Boines)
Georgetown University (Dr. Joseph Bellanti)
University of Vermont (Dr. George Schumacher)

Man Years:

Total:	3.3
Professional:	1.0
Other:	2.3

Project Description:

Objectives: To conduct comparative study of the role of delayed hypersensitivity, humor immunity and interferon in neurotropic infections. To study the development of the fetal immune system. To develop immunologic methodology for evaluating intrauterine infections and CNS infections. To investigate the immunopathology of Multiple Sclerosis and other neurologic diseases. To conduct serologic survey with spinal fluid and sera from patients with chronic diseases of the CNS.

Methods Employed: Rhesus monkeys were inoculated *in utero* with various viruses at the first, second, and third periods of gestation. At appropriate intervals, the fetuses were removed and blood collected for immunological evaluation. Antibody levels were determined by CF tests; lymphocyte transformation was measured by uptake of tritiated thymidine in the presence of virus antigens; interferon production was established by Sindbis HA inhibition assays. Concomitantly, the pathogenesis of mumps virus was studied in some fetuses to determine the fetal susceptibility at various stages of gestation and to correlate these observations with the immunological data.

Sera from patients with various chronic infections were selected for immunoglobulin analysis. The sera were precipitated by the ammonium sulfate method and concentrated ten times in saline. Then preparations were ultracentrifuged in linear gradients of sucrose and 7S gamma globulins separated from 19S molecules. The purified immunoglobulin fractions were serologically evaluated against different antigens to determine antibody specificity of early and late immune responses which might provide clues as to the nature of the causative agent. In addition, studies employing Sepharose columns were initiated to purify IgM from the sera prior to indirect immunofluorescent assays with specific anti-19S fluorescein-conjugated serum in tissue cultures infected with different agents. Since a constant residual amount of specific IgM seems to be associated with persistent viral infections and since the FA assay is the most sensitive serological procedure, it is conceivable that such approach could give us leads as to whether a given agent is associated with chronic infectious diseases.

Sera and spinal fluid from patients with MS were compared with specimens from patients with other neurological diseases using the immunodiffusion technique and neutralization tests. Highly concentrated measles virus antigens were utilized for the gel diffusion tests and the Edmonston strain of measles virus was employed for neutralization assays.

Lewis' rats were immunized with UV killed SSPE isolates and prototype measles virus combined with Freund's adjuvant and after antibodies developed and delayed hypersensitivity become manifest, these animals served as serum and lymphocyte donors to groups of histocompatible, non-immunized animals which were subsequently challenged with $10LD_{50}$ neurotropic measles virus.

Major Findings: Animal experiments indicated that great antigenic differences exist between the 5 SSPE isolates obtained in our laboratory. Some of the isolates were highly antigenic, eliciting extraordinary levels of HI and CF antibodies, whereas other strains proved to be poor antigens. Great variability was also found in their ability to induce delayed hypersensivitity.

Chronological evaluation of the antibody response of the fetal rhesus monkey following rubella virus inoculation showed that in the first period of gestation the fetuses are uncapable of synthesizing antibody. In the second and third periods good levels of antibody were obtained.

Measles-specific IgM was detected in the sera of SSPE patients. The macroglobulin was separated in Sepharose column and its specificity determined by HI test. This finding suggested that the suppressed measles encountered in brains and lymph nodes of patients produces a continuous antigenic stimulus.

Significance of the Program to the Institute: The successful development of a simple diagnostic test for SSPE now warrants further immunological studies with cerebrospinal fluid from patients with other neurological diseases in an effort to define antibody patterns of diagnostic value. Of utmost importance is the use of more sensitive (qualitative and quantitative) assays to study the increased gamma globulin levels encountered in the spinal fluid of MS patients. A careful serological investigation of the type and specificity of the immunoglobulins present in the brain of MS patients and appropriately matched controls could provide important clues to the possible infectious nature of the disease. On the other hand, an antibody mediated or autoimmune theory on the etiology of MS could also be investigated by virtue of purifying CSF gamma globulins followed by I^{131} labeling and inoculation into monkey brains. The animals would be killed at 8-hour intervals and autoradiography of the brain sections could conceivably show antibody-antigen reaction sites. Another approach would be a direct immunofluorescent assay using fluorescein-coupled CSF gamma globulin with MS brain tissue cultures.

Proposed Course of the Project: Special emphasis will be given to the study of the fetal immune system in an effort to understand the relationship between the maturation of the fetus immunological defenses and its resistance to transplacental infection. We will focus in the rhesus monkey model system as this non-human primate is known to be susceptible to intrauterine viral infections followed by severe malformations of the fetus.

Continued attempts to concentrate and identify specific gamma globulins from sera and spinal fluid of MS patients may prove of value in establishing whether or not an infectious agent is associated with MS.

Keyword Descriptors: Intrauterine infections, immune system and interferon, pregnant monkeys, neurotropic infections, antibody specificity.

Honors and Awards: None

Publications:

Oldstone, M. B., Bokisch, V. A., Dixon, F. J., Barbosa, L. H., Fuccillo, D. A., and Sever, J. L.: Subacute Sclerosing Panencephalitis: Destruction of Human Brain Cells by Antibody and Complement in an Autologous System. Clin. Immun. Immunopath. (In Press).

Barbosa, L. H., Palmer, A., London, W. T., Hamilton, R., Traub, R., and Ley, A.: Antibody Response of the Fetal Rhesus Monkey After Viral Infection. Proc. Soc. Exp. Biol. Med. (In Press).

Serial No. Z01 NS 02[illegible]34-03 ID
1. Infectious Diseases Branch
2. Section on Virology and Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Electron Microscope Membrane Studies of Measles and SSPE Viruses

Previous Serial Number: Same

Principal Investigators: Dr. M. Dubois-Dalcq, IDB, NINCDS

Other Investigators: Dr. T.S. Reese, NINCDS, K. Worthington, IDB, NINCDS

Cooperating Units: Section on Functional Neuroanatomy, Laboratory of Neuropathology and Neuroanatomical Sciences (NINCDS).

Man Years:

Total:	1.5
Professional:	1
Other:	0.5

Project Description:

Objectives: 1) To study structural changes in the menbrane of cells infected with different strains of SSPE virus, 2) To explore the differences between productive and non-productive SSPE infection.

Methods Employed: 1) Pellets of fixed cells were frozen in glycerol and freeze-fractured in a Balzer freeze-etcher apparatus. Replicas of the fractured membrane were made and studied under EM, 2) monolayers of cells on coverslips were fixed and dehydrated. They were then either air dried or critical point dried and a replica of their surface was made in the Balzer freeze-etcher. In some cases the surface viral antigens were labeled by the EM immunoperoxidase technique as described previously (J. Virol 12: 909-918, 1973), 3) Pellets of fixed cells were labeled with the immunoperoxidase method, frozen in water, fractured, and deep-etched in order to see both the inside and outside of the plasma membrane.

Major Findings: In the productive cells infected with a SSPE strain, early and late specific structural changes occurring in the cell plasmalemma of infected cells were identified. It was found that 1) surface antigens are at first diffusely distributed but subsequently move towards and become incorporated into strands, 2) viral buds begin to form when nucleocapsids underlying strands become attached to the membrane at sites manifested by small intramembranous particles. At this stage, regions of the modified membrane protrude to form ridges or bulges,

3) Nucleocapsid attachment sites, nucleocapsids and surface antigens then move laterally to be consolidated into continuous sheets of viral membrane, 4) Nucleocapsid attachment sites but not surface antigens are lost during maturation of detached virus.

In cells chronically infected with a SSPE strain, ridges on the surface were straight or hairpin shaped, rather than serpentine in productive cells, and were much longer than those in productive cells. Indeed, many villous processes extended from the cell surface, each with one or two hairpin ridges along its length. In thin sections, it was apparent that ridges are formed at contacts between tubules, thought to be nucleocapsid, and the cell surface. Thus it is possible that a single defect in the structure of the nucleocapsid prevents viral bud formation in the chronically infected cells.

Significance of the Program to the Institute: Immuno-freeze etching and surface replication combined with immunolabeling are new methods that allow studies of extensive areas of the membrane of infected cells as compared to controls. Using these methods, some mechanisms of the complete maturation of SSPE virus have been elucidated in productive cells, and found to be dramatically altered in cells latently infected with similar viruses. Similar events might take place in the SSPE brain but analysis of their structural and molecular basis can be more easily done *in vitro*.

Proposed Course of the Project: 1) Different strains of measles and SSPE virus will be studied with similar techniques (vaccine strain, wild strain, neurotropic strain, etc.) to explore the membrane characteristics of virus maturation in each case. Surface modification associated with cell fusion will also be studied, 2) Attempts to correct defective alignment or bud formation will be made *in vitro* using co-culture, cell sap, enzymes, etc., 3) Visna and scrapie virus will also be studied by freeze-fracture technique.

Keyword Descriptors: SSPE virus productive and non-productive infection *in vitro*, freeze-fracture, surface replication, deep-etching, immunoperoxidase labeling.

Honors and Awards: None

Publications: M. Dubois-Dalcq, J.M. Coblentz, A.B. Pleet. Subacute Sclerosing Panencephalitis. Unusual Nuclear Inclusions and Lengthy Clinical Course. *Archives of Neurology* 31: 355-363, 1974.

M. Dubois-Dalcq, K. Worthington, O. Gutenson and L.H. Barbosa. Immunoperoxidase Labeling of Subacute Sclerosing Panencephalitis Virus Antigen in Hamster Acute Encephalitis. *J. Neuropath. Exp. Neurol*. 34: 85-86, 1975.

M. Dubois-Dalcq, T.S. Reese, L.H. Barbosa, and D.M.D. Landis. Freeze Fracture Study of Vero Cells Infected with SSPE Virus - *J. Neuropath. & Expt. Neurol*. *34*: 87, 1975.

M. Dubois-Dalcq, K. Worthington, O. Gutenson, and L.H. Barbosa. Immunoperoxidase Labeling of Subacute Sclerosing Panencephalitis (SSPE) Virus in Hamster Acute Encephalitis. In press in Laboratory Investigation, 1975.

M. Dubois-Dalcq and T.S. Reese. Structural changes in the membrane of vero cells infected with Subacute Sclerosing Panencephalitis. Abstract "Perspectives in Multiple Sclerosis" February 10 and 11, NIH. In press in "Neurology", 1975.

Serial No. Z01 NS 02035-03 ID
1. Infectious Diseases Branch
2. Section on Virology and Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: EM Immunoperoxidase Studies in Multiple Sclerosis Brain

Previous Serial Number: Same

Principal Investigators: Dr. M. Dubois-Dalcq, IDB, NINCDS

Other Investigators: Dr. G. Schumacher, Vermont University, Burlington, Vt.
K. Worthington, IDB, NINCDS

Cooperating Unit: Vermont University, Burlington, Vermont
Department of Neurology

Man Years:

Total:	0.6
Professional:	0.3
Other:	0.3

Project Description:

Objectives: 1) To label under EM a possible measles viral antigen in the plaques of MS cases, 2) to precise the fine structural localization of the immunoglobulin IgG present in MS plaques.

Methods Employed: 3 patients were studied. Two to 6 hours after death, slices of brain from plaques areas were fixed in formaldehyde. Chopped sections were incubated in SSPE globulin coupled to peroxidase or in peroxidase labeled antihuman IgG and processed for EM.

Major Findings: Attempt to detect measles virus antigen in active MS plaques was done in 3 cases of which 2 displayed cells containing dense nuclear strands. In all 3 cases peroxidase labeled SSPE globulin did not stain dense strands or any other structures in the MS plaques. However, the same conjugate was able to stain SSPE viral antigen in nerve cells of chronic SSPE hamsters, when performed with Dr. K. Johnson in his laboratory (VA Hospital, San Francisco).

With peroxidase labeled antihuman IgG, intense labeling was obtained in all plaques studied in the 3 cases and was absent in control white matter. Most of the label was intracellular. Plasma cells and hypertrophied astrocytes were stained whereas the oligodendrocytes and myelin present in shadow plaques were spared. Under EM, the extensive parallel endoplasmic reticulum of cisternae of plasma cells were labeled as well as the ribosomes and gliofibrilles within the astrocytes. The specialized perivascular astrocytic membrane was also strongly labeled.

These results confirm that plasma cells are producing IgG in the plaques and raises the question of the possible role of hypertrophied astrocytes in the immunopathogenesis of the disease.

Significance of the Program to the Institute: Multiple Sclerosis is one of the most widespread neurological diseases among young people in this country and its etiology is still unknown. Recent reports have suggested that MS may be associated with a viral infection (measles, in some cases). This may induce an autoimmune demyelination process.

Proposed Course of the Project: When other MS brains will be available, the possibility of inactivation of measles viral antigen after death or of another viral antigen being present in the MS lesions should be explored. Also the significance of the IgG localization in the brain should be assessed by appropriate controlled studies.

With the help of the Neuroimmunology Branch from our Institute (Dr. McFarlin), CSF IgG and possibly brain IgG should be isolated, purified, concentrated and coupled to peroxidase. Normal and MS brain sections will then be incubated with this conjugate. The aim would be to determine against which antigen (viral or tissular?) are directed the antibodies apparently synthesized in brain.

Keyword Descriptors: Multiple Sclerosis, plaques, dense strands, EM immunoperoxidase, SSPE viral antigen, IgG synthesized in brain.

Honors and Awards: None

Publications: M. Dubois-Dalcq. Immunoperoxidase Studies on Multiple Sclerosis Brain. Abstract, "Perspectives in Multiple Sclerosis" February 10 and 11, NIH, in press in "Neurology",1975.

Serial No. Z01 NS 02036-03 ID
1. Infectious Diseases Branch
2. Section on Virology and Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunopathology of Chronic Neurologic Infections

Previous Serial Number: Same

Principal Investigators: Dr. Luiz H. Barbosa, IDB, NINCDS
Dr. Jerome E. Kurent, IDB, NINCDS
Dr. Monique Dubois-Dalcq, IDB, NINCDS

Other Investigators: Mr. Otto Gutenson, IDB, NINCDS
Mr. Leonard Moore, IDB, NINCDS

Cooperating Units: None

Man Years:

Total:	0.9
Professional:	0.4
Other:	0.5

Project Description:

Objective: To study the immunopathology of SSPE-like illness in hamsters. To establish the role of antibody and/or cellular immunity in the development of chronic neurotropic measles infection in the newborn hamsters. To evaluate the use of different chemotherapic agents on the treatment of SSPE-like syndrome of the hamster.

Methods Employed: Recent studies indicate that newborn hamsters with maternal antibody survive a lethal dose of HNT (Hamster Neurotropic measles virus) and, eventually a certain percentage of the animals develop a latent brain infection which resembles SSPE in man. The same phenomenon occurs with weaning hamsters, and it has been suggested that the presence of antibody associated with an immature cellular immunity is crucial for the production of the SSPE-like syndrome. This study proposes to manipulate these two components of the immune system in order to establish the roles of antibody and delayed hypersensitivity in the hamster model system.

Hyperimmunized animals served as donors of serum and splenocytes. Litters were divided into 4 groups of 5 litters each:

a) Virus alone
b) Virus and antibody
c) Virus and splenocytes
d) Virus and antibody and splenocytes

Conclusions will be drawn after a careful follow-up of all animals during which will be critical to distinguish between survivors with no evidence of virus and survivors undergoing suppressed measles brain infection (SSPE-like illness). This distinction must be done through immunofluorescence, H&E staining, EM and cocultivation.

The hamster model system is intended to be used to evaluate the effect of anti-viral drugs such as Ara-A, Ara-C, IUDR, and others.

Major Findings: Passive immunization protected 3 out of 10 animals and delayed the death of 7 out of 10 hamsters. Splenocytes alone protected 2/10 and prolonged the course of the disease in 8/10. The combination antibody + splenocytes appeared to be more effective protecting 5/10 hamsters and delaying death of 5/10. Survivors are presently under observation for clinical signs and antibody pattern. Based on results obtained with this pilot experiment, we will design further protocols to conduct a thorough analysis of the hamster SSPE-like model system.

Ribavirin (1-B-D-Bibofuranosyl-1,,2,4-Triazole-3-Carboxamine), a potential anti-viral substance was found to protect hamsters against SSPE measles virus. In vitro studies suggested that the drugs mode of action is probably due to interference with cellular DNA & RNA synthesis. Further studies with Ribavirin are being conducted.

Significance of the Program to the Institute: The understanding of the involvement of the immune system in the development of the newborn hamster SSPE-like illness is of critical importance. The application of this type of analysis will provide valuable information on the immunopathogenesis of chronic viral infections of the CNS and possible means of prevention and/or control of the disease in animals. These new insights into the causes of damage to the CNS will provide guidelines for new experimental therapeutic approaches for the human counterpart of the disease.

Proposed Course of the Project: The combined immunologic and virologic study will continue for at least one more year during which we plan to evaluate our methodology and specific testing. Once they prove satisfactory a larger number of animals will be utilized in the experiments in order to give us solid statistical interpretation of results.

Keyword Descriptors: SSPE hamster model system, chemotherapy, role of humoral and cellular immunity.

Honors and Awards: None

Publications: None

Serial No. Z01 NS 02069-02 ID
1. Infectious Diseases Branch
2. Section on Virology and Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cell Mediated Immunity and SSPE

Previous Serial Number: Same

Principal Investigator: Dr. Luiz H. Barbosa, IDB, NINCDS

Other Investigators: Mrs. Rebecca Hamilton, IDB, NINCDS

Cooperating Units: Dr. Michael Blaese, NCI

Man Years:

Total:	1
Professional:	0.5
Other:	0.5

Project Description:

Objectives: To develop an in vitro test to evaluate measles delayed hypersensitivity in SSPE patients.

Methods Employed: The Vr/60 SSPE chronically infected cell line will be used. This line has a characteristic CPE consisting of uniformly distributed small plaques. Cell-free virus has not been obtained under varying conditions, however, the nature of the cytopathic agent has been clearly defined as that of measles virus.

Lymphocytes from SSPE patients and normal individuals will be obtained by the Fycoll gradient method.

The target cells Vr/60 will be split and planted in petri dishes at a concentration of 2 x $10^{5.0}$. As soon as cells attach the media will be poured off and cultures overlayed with lymphocyte suspensions using a lymphocyte/target cell ratio of 10:1. These cultures will be placed onto a rocker platform and lymphocytes will be allowed to interact with Vr/60 cells for 24 hours. After this period of time the cultures will be refed with fresh medium; when control Vr/60 show typical plaques all petris will be fixed and stained and the procedure evaluated based on plaque counts.

Major Findings: Preliminary results indicated that the test is specific and sensitive. Initial work with lymphocytes from normal donors and SSPE patients suggested that these patients have normal cell-mediated immunity against measles virus.

Significance to the Program of the Institute: To date no reliable test for measuring measles delayed hypersensitivity exists. In addition, recent findings suggest a possible measles-specific deficiency of the cellular immunity in SSPE patients. The development of a plaque-reduction assay to evaluate measles cellular immunity will be of great value to give us a greater understanding of the immunopathogenesis of SSPE and thus permit new approaches to the prevention and treatment of the disease.

Proposed Course of the Project: At least 10-20 cases will be studied in parallel with normal measles-positive and measles-negative individuals. This test will also be used to evaluate the cellular immune capabilities of MS patients since this disease is suspected to be associated with an aberrant the degree of measles specific delayed hypersensitivity conferred following experimental administration of Transfer Factor.

Keyword Descriptors: Subacute Sclerosing Panencephalitis, Measles virus, Cell-mediated immunity, Plaque-reduction test, Fycoll-purified lymphocytes.

Honors and Awards: None

Publications: None

Serial No. Z01 NS 00972-04 ID
1. Infectious Diseases Branch
2. Section on Experimental Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Experimental Animal, Tissue Culture, Histopathological and Serological Investigation of the Role of Viruses and Other Micro-organisms in the Perinatal Period

Previous Serial Number: Same

Principal Investigators:
Dr. William T. London, IDB, NINCDS
Dr. Amos E. Palmer, IDB, NINCDS
Dr. John L. Sever, IDB, NINCDS
Dr. David A. Fuccillo, IDB, NINCDS

Other Investigators:
Mrs. Anita C. Ley, IDB, NINCDS
Mrs. Blanche Curfman, IDB, NINCDS
Mr. Robert L. Brown, IDB, NINCDS

Cooperating Units:
Dr. Stephen Kent
Department of Pathology
George Washington University
Washington, D.C.

Dr. Neal H. Levitt
Virology Division
U.S. Army Medical Research Institute of Infectious Diseases
Fort Detrick, Frederick, Maryland 21701

Man Years:

Total:	2.5
Professional:	1.5
Other:	1.0

Project Description:

Objectives: To study the role of viruses and other microorganisms in the perinatal period, the infection of gravid and nongravid animals of several different species by parenteral routes with various viruses and other microorganisms to determine the effects of these agents on the animals and their fetal tissues.

Attempt to recover inoculated agents from the various animals and fetal tissues and the correlation of these reisolations with gestational age at inoculation and dosage given. Relate these findings with gross and histopathological findings. Correlate all of this information with serological findings.

Methods Employed: An investigation of the role of viruses and other microorganisms in the perinatal period by the continual use of experimental animals, tissue culture techniques, histopathological studies, and serological testing.

Special quarantine facilities are maintained to prevent feral animals going through quarantine from becoming infected with endemic diseases such as rubeola and cytomegalovirus. A serologically defined breeding colony of Rhesus monkeys is maintained to provide susceptible pregnant females for inoculation.

Pregnant animals are being inoculated by various routes with viruses and other microorganisms, and held in isolation chambers throughout the experiment. These animals are being observed and serum samples, liver biopsy, spinal fluid and throat swabs are checked for evidence of disease and/or effects on fetal tissue. Pregnant animals will be delivered by cesarean section so all products of conception can be saved.

Virus isolation investigations utilizing tissue culture to recover viruses from tissues and fluorescent antibody technique to study the location of virus infection produced in experimental animals are being employed.

Major Findings: Several viruses of human origin or viral vaccines produced for human use have been found to cause anomalies in Rhesus monkey fetuses. Viruses or vaccines tested were Influenza A AICHI/2/68/WP strain, mumps virus wild #24 P_3 strain, Venezuelan Equine Encephalitis (VEE) Vaccine TC83, Western Equine Encephalitis (WEE), B628, clone 15 vaccine and Influenza type A, HK/68 vaccine.

All agents were intracerebrally inoculated into fetal Rhesus monkeys at approximately 100 days gestation. The monkeys were delivered at term by cesarean section and immediately processed for serological, viral and histopathological examination.

All fetuses developed antibody titers to agents they had been inoculated with. Persistent virus infection was not found in the fetuses. One exception was in a mumps infected fetus; virus was isolated 55 days postinoculation. Hydrocephalus, either bilateral and symmetrical or bilateral and asymmetrical was the common anomaly found in the fetuses regardless of viral agent inoculated.

Bilateral cataracts were also a common finding in fetuses inoculated with VEE. The control animals in all groups did not develop titers or anomalies.

Significance of the Program to the Institute: A program using experimental animals, tissue culture techniques, and histopathological investigations compliments the strict serological approach being used on human sera obtained from the Collaborative Study and thus balances the investigations of the role of viruses and other microorganisms in the perinatal period. It presents the direct means of investigation of these agents which may contribute to human perinatal pathology.

Proposed Course of the Project: Further studies using Reo I Virus, Dengue Virus II vaccine and yellow fever vaccine inoculated into pregnant monkeys are in progress. In addition, acute studies using Influenza A and VEE viruses are in progress. Varying the route of inoculation and time in gestation of inoculation of the various known teratogens are in the planning stage.

Keyword Descriptors:

Role of viruses and other micro-organisms in the perinatal period of experimental animals.

Honors and Awards: None

Publications:

London, W.T.; Kent, Stephen, G.; Fuccillo, David A.; Sever, John L. Influenza Virus as a Teratogen in Rhesus Monkeys. Nature (In press)

Serial No. Z01 NS 01986-04 ID
1. Infectious Diseases Branch
2. Section on Experimental Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1 1974 through June 30, 1975

Project Title: Intrauterine Inoculation of Fetal Monkeys With Tissues From Patients With Chronic Diseases and Infections

Previous Serial Number: Same

Principal Investigators: Dr. William T. London, IDB, NINCDS
Dr. Amos E. Palmer, IDB, NINCDS
Dr. Luiz H. Barbosa, IDB, NINCDS

Other Investigators: Dr. David A. Fuccillo, IDB, NINCDS
Dr. John L. Sever, IDB, NINCDS
Mrs. Blanche Curfman, IDB, NINCDS

Cooperating Units: None

Man Years:

Total	0.7
Professional:	0.2
Other:	0.5

Project Description:

Objectives: The study of chronic diseases, such as Subacute Sclerosing Panencephalitis, Creutzfeldt-Jakob disease, Amyotrophic Lateral Sclerosis, Progressive Multifocal Leukoencephalitis, and Multiple Sclerosis following the inoculation intracerebrally of fetal rhesus monkeys.

Methods Employed: In many diseases, the fetus and young are more susceptible than the adult. The possibility of exploiting this greater susceptibility of the neonate was the basis of the design to inoculate the fetuses of pregnant monkeys at 100 days gestation with CNS material taken from patients showing clinical signs of the above diseases. Animals are subsequently delivered by cesarean section and held in isolation chambers for 3 to 5 years. Animals are observed daily for abnormal signs. Sera and spinal fluid are collected for study every three months.

Major Findings: The inoculated animals are now 38-50 months of age, to date, no abnormal signs have been observed in any of the monkeys.

Surveys of sera and cerebrospinal fluid for elevated antibody titers to a battery of antigens revealed no differences between inoculated animals and controls.

Significance of the Program to the Institute: A short time ago, the hypothesis that chronic or subacute degenerative diseases of the nervous system might be transmissible would have been an unbelievable conception to most medically trained pathologists or neurologists. To date, using animal models, at least two chronic degenerative conditions seen in humans have been transmitted. As these diseases become more fully understood, we may have new ideas for the study of other degenerative diseases of the brain and central nervous system.

Proposed Course of the Project: Two animals from each disease group will be immunosuppressed with cyclophosphamide about 2 months prior to their scheduled euthanasia in an attempt to activate any latent infection, then all animals will be euthanized at 60 months of age and their tissues processed for histopathological examination. Tissues will be saved for electron microscopic examination as well as light microscope.

Keyword Descriptors: Intrauterine inoculation of monkeys with tissues from patients with chronic diseases such as Subacute Sclerosing Panencephalitis, Creutzfeldt-Jakob disease, Amyotrophic Lateral Sclerosis, Progressive Multifocal Leukoencephalitis, and Multiple Sclerosis.

Honors and Awards: None

Publications: None

Serial No. Z01 NS 01987-04 ID
1. Infectious Diseases Branch
2. Section on Experimental Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Caloric and Protein Restriction in Pregnancy and Their Effect on Measurements and Biochemistry of Newborn Rhesus Monkeys

Previous Serial Number: Same

Principal Investigators: Dr. William T. London, IDB, NINCDS
Dr. Amos E. Palmer, IDB, NINCDS
Dr. Donald B. Cheek, Dept. of Pediatrics,
Royal Children's Hospital
Victoria, Australia 3052

Other Investigators: Dr. John L. Sever, IDB, NINCDS
Dr. Jonas Ellenberg, OB, NINCDS
Dr. John G. Bieri, NIAID

Cooperating Units: Dr. Dominick Purpura
Albert Einstein Medical Center
Bronx, New York 10461

Dr. William Nyhan
University of California - San Diego Branch
P.O. Box 109
LaJolla, California 92037

Dr. Ernest Bueding
School of Hygiene
Johns Hopkins Hospital
Baltimore, Maryland 21205

Man Years:

Total:	0.6
Professional:	0.2
Other:	0.4

Project Description:

Objectives: Initiate a nutritional study of non-human primates using pregnant rhesus monkeys to test the hypothesis that there is no causal relation between maternal nutrition during pregnancy and certain sensory, pathological, immunological and biochemical characteristics of the infant. It is considered that a monkey trial in which the concept of randomness is permitted is a necessary preliminary to a human trial.

Methods Employed: In the nonhuman primate nutritional study, pregnant rhesus monkeys are maintained throughout pregnancy on one of three purified diets. One diet was restricted in protein, one deficient in both protein and calories, and the third was the control diet. The pregnant animals are delivered at 158 days gestation by cesarean section and the infant's tissues are processed for biochemical analysis. The nutritionally deprived female monkeys have been continued on their respective diets and studies for immunological responses to various antigens such as rubella-mumps virus vaccine, influenza virus, and Venezuelan Equine Encephalitis vaccine (TC83) will be carried out. The animals' immune functional ability will be tested using the antibody complement mediated cytotoxicity test.

Major Findings: Twenty-four viable fetuses were delivered from monkeys maintained on one of 3 diets:

Group 1 - Control diet	(8 fetuses)
Group 2 - Protein deficient-calorie sufficient diet	(8 fetuses)
Group 3 - Calorie deficient-protein deficient diet	(8 fetuses)

All organs were weighed at sacrifice and anthropometic measurements taken. When the fetal measurements (body weight, crown-rump, crown-heel and head circumference) were compared, no significant differences were found between the fetuses from monkeys maintained on the three diets.

No obvious differences were found in the neuronal development when a survey was made of a number of brain sections of fetuses from monkeys on the three different diets. It is impossible without extensive quantitative analysis to detect differences in dendritic growth patterns or dendritic spine distributions in these fetal brains.

Significance of the Program to the Institute: General obstetric practice in the United States is to restrict weight gain during pregnancy except where specifically contraindicated. Investigators analyzing the maternal weight gain/birthweight data from the Perinatal Research Program have concluded that this practice may be actively increasing the low birthweight rate, and possibly the perinatal mortality rate. Indeed, this practice may produce babies who are smaller than expected for gestation and who may be poor performers postnatally and of lower intelligence.

Proposed Course of Project: Statistical analysis is now being done on all of the data collected by Dr. Cheek's group in their biochemical assays of the fetal tissues. These results will then be reported. The completion of the immunological studies on the nutritionally deprived female monkeys will be done as outlined above.

Keyword Descriptors:

Caloric and Protein Restriction in Pregnancy, their effect on newborn Rhesus monkeys.

Honors and Awards: None

Publications: None

Serial No. Z01 NS 01988-04 ID
1. Infectious Diseases Branch
2. Section on Experimental Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Herpesvirus Induction of Cervical Cancer in Cebus Monkeys

Previous Serial Number: Same

Principal Investigators: Dr. Amos E. Palmer, IDB, NINCDS
Dr. William T. London, IDB, NINCDS
Dr. Andre J. Nahmias
Dept. of Pediatrics, Emory University
School of Medicine, Atlanta, Georgia 30303
Dr. John L. Sever, IDB, NINCDS

Other Investigators: Dr. David Fuccillo, IDB, NINCDS
Mrs. Renee Traub, IDB, NINCDS

Cooperating Units: Dr. David Sly
Meloy Laboratories
Springfield, Virginia

Man Years:

Total:	1.2
Professional:	0.7
Other:	0.5

Project Description:

Objectives: Numerous reports suggest a relationship between infection with Herpes Simplex Virus, Type 2, (HSV-2), and cervical cancer. The cebus monkey responds to infection with HSV2 quite similarly to man. One important way to establish causal relationship between genital herpes infection and cervical cancer would be to determine whether cervical cancer can be produced in monkeys that are infected by genital inoculation with herpes simplex type II virus.

Methods Employed: A total of 225 female cebus monkeys receive inoculations with HSV2 and 75 receive inoculations with control material at 6-month intervals. Females are housed with males to produce pregnancies which are considered to be a part of the pathogenesis of cervical carcinoma in humans. All animals are closely monitored for evidence of infection and development of cervical anaplasia.

Major Findings: Persistent reinoculation of female cebus monkeys with HSV2 results in the infection of approximately 50 percent at each inoculation.

All animals have become infected one or more times after 3 inoculations. As in humans, a low incidence of spontaneous recurrence of HSV2 infection occurs with virus shedding and genital lesions. Venereal spread from female to male occurred in approximately 18 percent of the males with development of serum antibodies and penile lesions. Virus shedding from infected males occurs at low incidence. HSV2 was isolated from the dorsal root lumbo-sacral ganglia of a virus infected cebus monkey by explant cocultivation with MA184 tissue culture.

Five virus infected females have developed persistent cytological changes of dysplasia and 8 animals show persistent changes of atypia in cervical smears. No control animals show persistent changes at this time.

Colposcopic evaluation permits the examination of the cervix of the cebus monkey with magnification, revealing surface and vascular changes not detectable by the naked eye. Minor differences between the anatomy of the female cebus genital tract and that of the human make interpretation of changes difficult. Animals are examined at 6-month intervals and photographic record are made for each animal.

Because pregnancy is believed to be a factor in the pathogenesis of cervical carcinoma in the human, males are caged with females at ratios of 1:1 to 1:3. A total of 55 pregnancies have occurred to date. Of these, 21 have delivered with 14 live births and 8 abortions. HSV2 has not been isolated from either neonates or aborted fetuses.

Significance of the Program to the Institute: The role of HSV2 infection in perinatal diseases, cervical carcinoma and chronic neurological diseases of neonate humans stimulated research to develop a nonhuman primate model for studying the pathogenesis and oncogenic potential of this virus.

Proposed Course of the Project: The persistent reinoculation of female monkeys at 6-month intervals and virological, cytological, serological and clinical evaluations will continue. Animals which develop cytological changes of anaplasia will be examined monthly to determine if persistence and progression occur. The study should continue until all animals have been studied for a 5-year period. Additional tests for the detection of neoplasia will be performed as they become pertinent.

Keyword Descriptors:

Herpes Simplex Virus Type II Induction of Cervical
Cancer in Cebus Monkeys

Honors and Awards: None

Publications:

Palmer, A.E., London, W.T., Nahmias, A.J., Naib, Z.M., Fuccillo, D.A., Tunca, J., Ellenberg, J.H., and Sever, J.L. Investigation of the Oncogenic Potential of Herpes Simplex Virus, Type 2, in Cebus Monkeys: A Preliminary Report. In Press - Cancer Research.

Serial No. Z01 NS 01989-04 ID
1. Infectious Diseases Branch
2. Section on Experimental Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Transmission of Hepatitis B Virus to Subhuman Primates

Previous Serial Number: Same

Principal Investigators: Dr. William T. London, IDB, NINCDS
Dr. Robert H. Purcell, IDB, NIAID

Other Investigators: Mrs. Blanche Curfman, IDB, NINCDS

Cooperating Units: None

Man Years:

Total:	0.9
Professional:	0.2
Other:	0.7

Project Description:

Objectives: The study and characterization of Hepatitis A and B in non-human primates.

Methods Employed: Different species of non-human primates are inoculated (orally or parenterally) with material containing hepatitis A or B agents. The animals are monitored for serological, biochemical, and histopathological evidence of infection.

Major Findings:

Hepatitis A: Hepatitis A virus has been successfully transmitted to marmosets and chimpanzees; infection results in biochemical and histopathological evidence of hepatitis in both species. Since both chimpanzees and marmosets are expensive and difficult, if not impossible, to obtain, we are attempting to transmit hepatitis A virus to other species of non-human primates. Serum samples from six species of primates (rhesus monkeys, patas monkeys, African green monkeys, baboons, cebus monkeys, owl monkeys) have been tested for anti-HA by immune electron microscopy and/or immune adherence. One or more animals of the following species were found to have such antibody: rhesus monkey, owl monkey, patas monkey and African green monkey. Seronegative representatives of each of these species tested, plus other species as they become available for testing, are being inoculated with hepatitis A virus and being monitored for serological, biochemical, and histopathological evidence of infection. Rhesus monkeys developed serologic evidence of infection in a preliminary study, but we have been unable to confirm this in a subsequent study. Results of studie on other non-human primates are not yet available.

Hepatitis B:

Transmission to Rhesus monkeys: We have been unable, during the past year, to expand our studies of hepatitis B virus infection in rhesus monkeys because of an inability to demonstrate successful transmission in recent experiments. The reason for this problem is unclear, but may be related to loss of infectivity of existing pools of virus and/or diminished susceptibility of rhesus monkeys available for testing. Attempts to resolve this problem are in progress.

Transmission to Chimpanzees: Pools of hepatitis B virus subtype ayw and adw were both found to have infectivity titers of approximately $10^{7.5}$ chimpanzee infectious dose 50 units per ml. A pool of HBV of subtype adr has an infectivity titer of at least 10^6, and a pool of subtype ayr has a titer of greate than 10^1 but less than 10^4. Additional titration studies of these latter tw pools are still in progress. Preliminary results of cross-challenge experiments with these pools in chimpanzees indicates infection with one subtype of HBV confers partial, if not complete, protection against rechallenge with another subtype.

Immunoprophylaxis:

Passive Immunization: The protective effect of high titered hepatitis B immune serum globulin is being evaluated under controlled conditions in chimpanzees inoculated with known doses of HBV. Preliminary experiments indicate little, if any, protective effect of hepatitis B immune serum globulin against HBV infection or illness, but additional studies with more chimpanzees must be carried out.

Non-A, Non-B hepatitis: Serum samples from patients acutely or chronically ill with non-A, non-B hepatitis have been inoculated into chimpanzees in an attempt to transmit an etiologic agent. The animals did not demonstrate histopathological or biochemical evidence of hepatitis. However, we cannot rule out an unapparent infection, since serologic methods of diagnosis are still not available.

Significance of the Program to the Institute: Infectious Hepatitis (Hepatit A) and Serum Hepatitis (Hepatitis B) remain two of the major human viral diseases for which adequate control measures have not been developed. This is so because, despite heroic efforts, neither virus has been isolated in tissue culture; and, until recently, neither virus could be transmitted to laboratory animals in which it could be studied. Although some important information was obtained through the use of human volunteer studies during the 1940's and 1950's, an appreciation of the potential danger of such stud s, coupled with changing concepts of medical ethics, has greatly curtailed the experimental transmission of viral hepatitis in volunteers. This study developed in part as the result of serological surveys in the perinatal research program.

Proposed Course of the Project: The ultimate goal of our hepatitis studies is the control of hepatitis A and hepatitis B through passive, or, preferably, active immunization. Passive immunization for the prevention of hepatitis A is an accepted medical fact. Passive immunization for the prevention of hepatitis B is more controversial, but studies to determine its efficacy can and are being carried out in human populations. However, attempts to develop vaccines against hepatitis A and hepatitis B must be carried out, to the greatest extent possible, in animals; and it is to this purpose that much of our effort in animal studies will be put.

Keyword Descriptors:

Hepatitis A and B in non-human primates

Honors and Awards: None

Publications:

Jules L. Dienstag, Stephen M. Feinstone, Robert H. Purcell, Jay H. Hoofnagle, Lewellys F. Barker, William T. London, Hans Popper, John M. Peterson, and Albert Z. Kapikian. Experimental Infection of Chimpanzees with Hepatitis A Virus. J. of Inf. Diseases (In press).

Lewellys F. Barker, James E. Maynard, Robert H. Purcell, Jay H. Hoffnagle, Kenneth R. Berquist, William T. London, Robert J. Gerety and Donald H. Krushak. Hepatitis B Virus Infection in Chimpanzees: Titration of Subtypes. J. of Inf. Diseases (In press).

Serial No. Z01 NS 02037-03 ID
1. Infectious Diseases Branch
2. Section on Experimental Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Perinatal Carcinogenesis in Erythrocebus Patas Monkeys

Previous Serial Number: None

Principal Investigators: Dr. William T. London, IDB, NINCDS
Dr. Amos E. Palmer, IDB, NINCDS
Dr. Jerry Rice, EPB, NCI

Other Investigators: Mrs. Blanche Curfman, IDB, NINCDS

Cooperating Units: Dr. David Sly, Meloy Laboratories, Springfield, Virginia

Man Years:

Total:	0.8
Professional:	0.3
Other:	0.5

Project Description:

Objectives: To establish a non-human primate model for chemical transplacental-perinatal carcinogenesis. Then use this model to study the greater sensitivity of fetal and neonatal animal tissues to chemical carcinogens, as well as the role of factors other than the carcinogens themselves in enhancing or suppressing chemical carcinogenesis in experimental animals during intrauterine life and infancy.

Methods Employed: Pregnant patas monkeys will receive single or repeated injections of 0.1 mmoles/kg of 1-ethyl-1-nitrosourea (ENU), either intravenously in pH3 isotonic citrate buffer or intraperitoneally in trioctanoin. The compound will be administered at various times in gestation.

Major Findings: Transplacental carcinogenesis studies in the E. patas monkeys have shown that pregnant animals and their fetuses tolerate up to 6 sequential intravenous injections of ENU, at a single-dose level of 0.1 mmole ENU/kg maternal body weight. This total dose is at least 10 times that necessary to induce a 100 percent incidence of nerve tumors in rats. However, no tumors of any kind have yet been observed in monkeys exposed to ENU in utero and observed up to 24 months after birth. Synthesis of ENU-ethyl-^{14}C is now in progress, to be used in defining the maternal/fetal distribution, tissue binding, and elimination of ENU from the patas in comparison with the highly susceptible Sprague-Dawley rat. Comparative teratogenic effects of ENU in these species are being evaluated.

Significance of the Program to the Institute: Research for animal models of human childhood neoplasms should provide an insight into the types of causative agents and modes of exposures responsible for childhood cancer. It is to be expected that natural selection would tend to eliminate genotypes in the human population which predispose individuals to the development of fatal neoplasms before attaining reproductive age, yet the incidence of tumors in childhood is high. Epidemiological studies have pointed to the occurrence of childhood neoplasms in association with certain types of congenital malformation which are non-inherited, and suggest that environmental agents, alone or in combination, may play a role in the induction of such neoplasms.

Most tumors induced transplacentally in rodents are of adult types and appear during adult life in individuals exposed in utero, resembling human experience with diethylstilbestrol. The ENU studies in patas monkeys will provide experimental data indicating whether adult or pediatric tumor types are more likely to develop in at least one species of primate in response to carcinogenic exposure in utero, and thus whether chemical carcinogens are likely to be involved in the genesis of pediatric or adult types of tumors in man.

Proposed Course of Project: Patas monkeys exposed to ENU either transplacentally or directly (after birth) should continue to be observed for the development of tumors. Pharmacodynamic studies will be initiated to compare maternal-fetal distribution and tissue/organ localization of ENU-^{14}C in this species, versus the rat and mouse. Comparative dose-response curves for ENU-induced teratogenic effects in patas monkeys and Sprague-Dawley rats will be carried out as a rapid measure of relative susceptibility to this compound.

Keyword Descriptors:

Perinatal Chemical Carcinogenesis in
Erythrocebus Patas monkeys using 1-ethyl-1-
nitrosourea (ENU)

Honors and Awards: None

Publications: None

Serial No. Z01 NS 02068-02 ID
1. Infectious Diseases Branch
2. Section on Experimental Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Simian Hemorrhagic Fever in Patas and Rhesus Monkeys

Previous Serial Number: None

Principal Investigators:	Dr. William T. London, IDB, NINCDS Dr. Amos E. Palmer, IDB, NINCDS Dr. John L. Sever, IDB, NINCDS
Other Investigators:	Dr. David A. Fuccillo, IDB, NINCDS Dr. David Madden, IDB, NINCDS Dr. Hilton B. Levy, LVD, NIAID Mrs. Blanche Curfman, IDB, NINCDS Mr. Robert L. Brown, IDB, NINCDS

Man Years:

Total:	1.1
Professional:	0.5
Other:	0.6

Project Description:

Objectives: Simian hemorrhagic fever (SHF) is an acute, highly fatal disease which occurs in severe epizootics among Asian macaque monkeys. The virus responsible has been found in blood serum from clinically normal African monkeys. Under certain conditions one strain of the virus causes chronic disease among patas monkeys (Erythrocebus patas). This strain of the virus has not been grown in cell or tissue culture, thus its presence can only be determined by the inoculation of rhesus monkeys (Macaca mulatta) who develop a rapidly progressing disease that is indistinguishable from that caused by other strains of SHF virus.

A reliable serological test to detect both antibodies and antigen is needed to identify infection in the feral patas monkey. There are at least three strains of SHF virus. One strain (LVR) has been isolated in cell culture from epizootics in Rhesus monkeys at NIH and at Sukhumi, USSR, in 1964.

Strain involved in the 1972 rhesus monkey and 1973 patas monkey outbreaks at NIH have not been isolated in cell culture. This must be done before the agents can be conclusively identified. This disease presents an infectious disease model in which resembles 1) immune complex diseases (such as lymphocytic choriomenengitis (LCM), 2) disseminated intravascular coagulopathy (DIC), and 3) the hemorrhagic fevers of man. We intend to study SHF in patas and rhesus monkeys in an attempt to better characterize its pathogenesis, prevention and treatment.

Methods Employed: There is presently no procedure available to detect the carrier state in clinically normal patas monkeys other than the inoculation of rhesus monkeys. We are making progress toward the development of laboratory diagnostic techniques for this purpose. We have attempted immunodiffusion, complement fixation, and radioisotope assays.

We are also exploring the following aspects of the disease:

1. Locate the site (organs or tissues) responsible for the replication of the virus in the chronically infected patas monkey.

2. Determine whether there is a virus-antibody complex in the serum of infected patas monkeys and, if so, whether this complex is responsible for the disease.

3. Continue attempts to cultivate the causative agent on cell or tissue culture, and other animals such as nude mice.

4. To evaluate immunosuppressive agents, corticosteroids, and anticoagulants as therapeutic agents to prevent or reverse the course of the disease.

5. Attempt to develop and test inactivated antigens as immunizing agents to protect animals against a challenge of virulent virus.

Major Findings: Simian hemorrhagic fever is an endemic disease in feral E. patas monkeys (10%). The agent has been found occasionally in feral Papio cynocephalus and Cercopithecus aethiops monkeys.

The disease among Asian Macaque monkeys is caused by a laboratory accident, usually transmitted by contaminated needles. Once the disease is established in a Macaque colony, it is readily transmitted to other monkeys by direct contact, fomites, aerosol, or parenteral inoculations.

In the natural host, E. patas and other African species, the virus is not transmitted by direct contact. Parenteral administration must be used to transmit the disease among these species.

A highly specific antibody against SHF has been produced in guinea pigs using antigen in sera of feral E. patas monkeys. This reacts in a complement fixation test with antigen in the sera of acutely ill Rhesus monkeys as well as chronically infected E. patas and Cercopithecus aethiops monkeys. The tissue culture produced antigen does not react with antisera from feral monkeys. However, one passage of this virus in Rhesus monkeys results in a reactive antigen in the sera of the monkey. This indicates that the tissue culture adapted strain of virus has lost some antigenic components. Furthermore, antisera produced in guinea pigs using tissue culture adapted virus reacts only with the homologous strain of virus.

Polyinosinic polycytidylic acid (Poly-I-poly C complex) is an effective interferon inducer and antiviral agent in Rhesus monkeys. The complex can be used prophylactically to protect Rhesus monkeys from a lethal dose of SHF virus. However, it is not effective as a therapeutic agent for this disease.

Significance of the Program to the Institute: SHF offers a potential animal model which may permit research on several diseases of significance to humans.

Proposed Course of the Project: Studies in patas monkeys will include virus cultivation attempts in cell cultures, attempts to isolate and identify the virus-antibody complex and to demonstrate its presence in affected organs of infected animals, evaluation of immunosuppressants, corticosteroids and anticoagulants as therapeutic agents and evluation of the convulsions as to their cause and treatment.

Studies in Rhesus monkeys will involve testing therapeutic as well as immunoprophylactic agents.

Keyword Descriptors:

Simian Hemorrhagic Fever in Patas and Rhesus Monkeys.

Honors and Awards: None

Publications: None

Serial No. Z01 NS 02136-01 ID
1. Infectious Diseases Branch
2. Section on Experimental Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Clinical Studies with Experimental Animals Using Vaccines and Chemotherapeutic Agents for the Prevention and Control of Infectious Diseases.

Previous Serial Number: None

Principal Investigators: Dr. William T. London, IDB, NINCDS
Dr. Amos E. Palmer, IDB, NINCDS

Other Investigators: Dr. David A. Fuccillo, IDB, NINCDS
Dr. John L. Sever, IDB, NINCDS
Dr. Jerry M. Rice, EPB, NCI
Mrs. Blanche Curfman, IDB, NINCDS
Mr. Robert L. Brown, IDB, NINCDS

Cooperating Units: None

Man Years:

Total:	1.2
Professional	0.4
Other	0.8

Project Description:

Objectives: To study new prophylactic and therapeutic drugs for the prevention and control of infectious diseases. Safety testing candidate vaccines as to their immunogenicity and communicability in experimental animals.

Methods Employed: New chemotherapeutic agents which appear to warrant further investigation are studied in the appropriate experimental animals.

The animals are inoculated with a known infectious disease and then a therapeutic regimen is started using the new drug. Additional animals are prophylactically treated with the drug and then challenged with the infectious agent.

New vaccines as they become available will be safety tested in susceptable experimental animals. Animals will be vaccinated and caged with sentinel animals to determine communicability. The vaccinated animals will be challenged at appropriate times to establish the immunogenicity of the vaccine.

Major Finding: This is a new project and it is too early to report any findings at this time.

Significance of the Program to the Institute: Experimental animal studie provide the basic data necessary to evaluate potential chemotherapeutic agents and vaccines. They also provide information on the expected cours of infection in man. This information, gained from experimental animal studies, provides the necessary bridge to the implementation of clinical studies in man.

Proposed Course of the Project: The additional studies will be performed utilizing two new chemotherapeutic agents:

1. Phosphonoacetic Acid (PAA) topically administered to cebus monkeys infected with genital herpes.

2. Ribavirin, another agent, will be used on treated monkeys showing neurological signs following inoculation with subacute sclerosing panencephalitis virus.

Keyword Descriptors:

Testing new vaccines and chemotherapeutic agents in experimental animals.

Honors and Awards: None

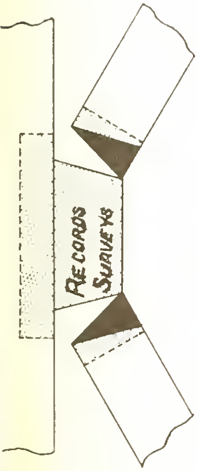
Publications: None

HOW TO USE
THESE SEPARATORS

Use one page for each separation.

Select appropriate tab, add further identification if desired, and cover it with scotch tape.

Cut off and discard all tabs except the one covered by tape.



TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

ANNUAL REPORT
July 1, 1974 to June 30, 1975
Neuroimmunology Branch
National Institute of Neurological and Communicative Disorders and Stroke

Dale E. McFarlin, M.D., Chief

The Neuroimmunology Branch was officially established in August of this year. The members of the staff began in December 1974 and the Branch Chief began February 1. Thus far, the Branch efforts have been largely administrative and have been directed toward recruiting personnel, acquiring and designing space, and ordering equipment. Three laboratory modules and two office modules have now been renovated. The remainder of the assigned space has been designed. Ten appointments have been made, and orders for the majority of the major equipment required by the Branch have been placed. Preliminary laboratory efforts have been devoted to setting up routine immunological and immunochemical techniques such as immunofluorescence, radioimmunoassay, lymphocyte identification, and specific lymphocyte transfer.

During the forthcoming year, it is anticipated that the scientific activities will focus on the following areas:

1. Assessment of immune response following virus infections. It is now known that the immune response is complex and consists of cell-mediated immunity and humoral immunity mediated by antibody. In addition, there are a number of control mechanisms such as blocking antibody, suppressor T-cells, and helper T-cells. It is our plan to study these various influences during the course of virus infections in experimental animals. Viruses are composed of multiple antigens and either whole, purified virions or preparations containing a restricted number of antigens will be used in the studies. Hence an important effort of the Branch will be to purify and characterize antigens from viruses and membranes of cells in the nervous system. These antigens will also be used for immunological studies of patients with neurologic diseases.

2. Control and regulation of the immune response. The mechanisms which control the immune response are important aspects of immunologically mediated disease and host resistance against infectious agents. Two examples of regulatory factors which will be studied in greater detail are:

A. Recurrent EAE. Lewis rats challenged with guinea pig basic protein develop EAE and lie moribund for 3-4 days; the majority subsequently recover and go on to have a second episode of CNS involvement. (D. McFarlin _et al_., _J. of Immunol_. 113: 712, 1974). The mechanisms responsible for both recovery following the 1st episode and the 2nd attack are unknown and have immediate relevance to clinical conditions characterized by spontaneous exacerbations and remissions.

B. Mice infected with measles have impaired function of thymus derived lymphocytes (H. McFarland, _J. of Immunol_. 113: 1978, 1974). The observation that measles infection can suppres a specific portion of immune response will be studied in greater detail.

3. Use of immunological techniques to identify cellular components of the nervous system. Preparations of neurons, oligodendroglia, and astrocytes will be obtained and used to immunize animals in hopes of obtaining antisera specific for each of these cell types. Such antisera will be used to study the distribution and appearance of surface antigens on cells in normal and pathological nervous systems. It is believed that antigens on the cell surface may play a role in CNS differentiation.

4. Study of the myelin membrane. A combination of immunological and chemical techniques will be used to study myelin and the various components of myelin in normal and diseased states. Experimental allergic encephalomyelitis will be used as a disease model to study alterations in myelin occurring as the consequence of immune mediated disease.

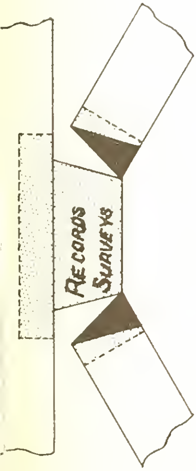
5. Assessment of the immune functions in patients and animals with chronic neurological diseases. It is anticipated that the Branch will conduct some clinical research. These studies for the most part will be delayed until laboratory facilities are fully developed. However, in time it is anticipated that the immune response to the nervous system antigens, viral antigens and other common antigens used to assess immune functions will be characterized in patients with neurological diseases. In particular, multiple sclerosis, SSPE and parkinsonism will be studied. Investigations of spinal fluid immunoglobulins and lymphocytes will be carried out. In additio patients will be characterized according to histocompatibility antigens, specified by both the HLA and the LAD loci. The results of such findings will be coordinated with other immune functions. Preliminary aspects of these studies have begun in collaboration with the Infectious Diseases Branch, IR and the Neurology Department at Johns Hopkins University.

HOW TO USE
THESE SEPARATORS

Use one page for each separation.

Select appropriate tab, add further identification if desired, and cover it with scotch tape.

Cut off and discard all tabs except the one covered by tape.



TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

ANNUAL REPORT
July 1, 1974 through June 30, 1975

Acting Associate Director, Collaborative and Field Research
National Institute of Neurological and Communicative Disorders & Stroke
National Institutes of Health

Goals of the Collaborative and Field Research (C&FR) Program of NINCDS has been to conduct directed research of importance and relevance in the fields of neurological diseases and communicative disorders. This program has attempted to fill the gaps in research areas of importance to NINCDS which are not covered by either the Intramural or Extramural programs of the Institute. The major part of this program has been implemented chiefly by means of contracts plus some in-house effort.

Due to a projected reorganization of our Institute to be effective approximately July 1, 1975 the Collaborative and Field Research area will be abolished and its functions taken over by four (4) program directors: Fundamental Neuroscience Program, Communicative Disorders Program, Neurological Disorders Program, Stroke & Trauma Program. This has resulted in future planning for these program areas only, rather than for a Collaborative and Field Research program.

The contracts budget for the Collaborative and Field area for FY 1975 was $8,000,012 and involved 27 new contracts and additional funding for on-going contracts in the amount of $4,225,676. In addition, $1,000,618 was spent for in-house research. These funds were somewhat less than had been hoped for but were adequate to carry out existing programs and start some additional programs. The minimal increase in other objects monies was spent essentially for new equipment for the reading of microfilms in our Perinatal Research Branch and for scientific equipment in our Pharmacology and Head Injury & Stroke Laboratories. There have been several program changes in the Collaborative and Field area during FY 1975. During early FY 1975, the Infectious Diseases Branch was transferred to the Intramural Research area. This branch was originally a section under the Perinatal Research Branch, which was organized primarily to process specimens from the collaborative perinatal study. As this study nears completion other areas of research more allied to the Intramural Research area were being taken on by the Infectious Diseases Branch and it was determined that it should be moved at this time.

Dr. Jacob Brody, Chief, Epidemiology Branch was transferred to the Office of the Director, Intramural Research and detailed to the Atomic Bomb Casualty Commission at Hiroshima, Japan. Dr. Roswell Eldridge was appointed Acting Branch Chief and the on-going contracts of this area were transferred to Mr. William Weiss, Chief, Office of Biometry, C&FR. The laboratory operation of the Epidemiology Branch consisting of Dr. Lon White and Dr. George Nemo and staffs was transferred to the Laboratory of Central Nervous Systems Studies, Intramural Research. The Section on Genetics in Epidemiology will be transferred prior to the end of FY 1975 to the Infectious Diseases Branch, Intramural Research. Other functions of the Epidemiology Branch, in the upcoming reorganization, will be transferred to the Office of Biometry, Office of the Director, NINCDS. The Guam field station operation has been transferred to the Office of the Director, Intramural Research and operation and direction of this facility will continue from that office. In February 1975, the Director, NINCDS created an Ad Hoc Scientific Evaluation Branch from components of the Scientific Evaluation Unit, Research Contracts Section, C&FR and the Special Program Branch of the Extramural programs.

During FY 1975 several major program moves were made within the Collaborative and Field area which have led to major disruption of our programs, but which are now again beginning to function effectively. The Office of the Acting Associate Director, the Administrative Office, and the entire Research Contracts Section were moved to adjoining space on the 7th floor of the Federal Building. In addition, the entire Applied Neurologic Research Branch, with the exclusion of the Pharmacology Laboratory in Building 36 and the Head Injury & Stroke Section in Building 36, were moved to the 1st floor of the Federal Building. This was a major rearrangement and the television engineering of this branch is still not in full operation due to environmental difficulties in the Federal Building. Hopefully these difficulties will be overcome early in FY 1976 and this branch can again become more productive. With the exception of the ANRB move, all new space in the Federal Building was required to be absorbed from existing space of other C&FR programs. This has required extensive rearrangement in our Perinatal Research Branch and our Office of Biometry operations.

Another source of concern during the year was the requirement that we vacate our Epidemiology Branch space on the 10th floor of the Federal Building and consolidate other space in order that the entire roof of the Federal Building could be removed and replaced. We are hopeful that this space will be returned to us during FY 1976.

Dr. W. Watson Alberts has continued throughout this Fiscal Year as Acting Head, Section on Biomedical Engineering and Instrumentation. This section sponsors directed research projects for the purpose of developing instrumentation needed to advance the diagnosis and treatment of neurological and sensory disorders. The section will be abolished in the reorganization and the functions transferred to the Fundamental Neurosciences Program.

The Communicative Disorders Section, soon to be transferred to the Communicative Disorders Program area, has greatly expanded their area of responsibility and the number of contracts which they sponsor. In December 1974, a staff fellow, Dr. Edward Cudahy, joined the program and was assigned to intramural Psycho-acoustic research.

Our Applied Neurologic Research Branch continues under the guidance of Dr. J. Kiffin Penry. The Epilepsy Section of this branch will, in the reorganization, be transferred to the Neurological Disorders Program and the Head Injury Section will be transferred to the Stroke and Trauma Program. During FY 1974, NINCDS funded eleven studies to determine the feasibility of successfully establishing a Comprehensive Epilepsy Program. During FY 1975, it is expected two contracts will be negotiated for the actual establishment of Comprehensive Epilepsy Programs. In the Head Injury & Stroke Section, the NINCDS Collaborative Study of Cerebral Survival (the brain death survey) was brought to a close during FY 1974 and a final report of the program director was received by the Institute, editorally reviewed, and several major scientific reports are in press or in preparation. The section is involved in [illegible] and in conduct of studies of controlled hypertension in coma, [illegible] laboratory [illegible] experimental animals as a prelude of protocols proposed for clinical investigations in humans.

As previously stated the Epidemiology Branch, OBEP, in the reorganization will be combined with the Office of Biometry, under the Office of the Director, NINCDS, with the Section on Genetics in Epidemiology being transferred during this fiscal year to the [illegible] Branch, Intramural Research. The Genetics Section in this fiscal year has been instrumental in assisting in the organization of an international conference dealing with the topic of [illegible] ([illegible]) [illegible] covering [illegible] the establishment of [illegible] Information Centers [illegible] Epidemiology, [illegible] The [illegible] conferences [illegible] of the [illegible] Information Centers. [illegible] Information Center for Hearing, Speech and Disorders of Human Communication, [illegible] The Clinical Neurology Information Center, University of Nebraska, [illegible] by the National Advisory Council. [illegible] were transmitted to the Institute's Scientific Information Program Advisory Committee, which provided recommendations to the Institute regarding the operation of each of these centers.

The Office of Biometry, which will soon be transferred to the Office of the Director, NINCDS, is a central resource to NINCDS for research and statistical [illegible], computer programming, systems analysis and data processing. In addition, [illegible] collection.

In the Collaborative Perinatal Research area, data collection has now been completed and the [illegible] of the project has [illegible] to data analysis and interpretation for publication of results of the Collaborative Perinatal [illegible] [illegible] in the field of [illegible].

T TO USE
ESE SEPARATORS

e one page for
n separation.

ect appropriate
, add further
ntification if
ired, and cover
with scored
e.

off and discard
tabs except the
covered by tape.

SSED SEPARATOR SHEET
rm PER-69
-56

ANNUAL REPORT
July 1, 1974 through June 30, 1975
Communicative Disorders, Collaborative and Field Research
National Institute of Neurological and
Communicative Disorders and Stroke

Introduction:

The program on Communicative Disorders was established in the Office of the Associate Director for Collaborative and Field Research in April, 1972 to develop and carry out research directed toward the improvement of diagnosis and treatment of disorders of hearing, speech, and language. The scope and extent of Communicative Disorders activities has greatly expanded since September, 1973 with the arrival of a new program head, since June, 1974 when a staff member responsible for research on speech and language disorders joined the program, and since December, 1974 when a staff fellow was assigned to intramural psychoacoustics research. Whereas the program originally emphasized hardware devices to assist the deaf, there is now an ongoing contract and intramural research program concerned with a variety of communication problems.

Currently, the responsibilities of the Communicative Disorders program are:

1. Planning and conduction of directed research having the goal of diagnosis, treatment, amelioration, and prevention of communicative disorders.

2. Coordination of NINCDS research on noise with other agencies both within and outside the Department of Health, Education and Welfare, including the Environmental Protection Agency.

3. Serving as a source of scientific expertise and consultation for topics concerning communicative disorders.

Directed Research: Program planning of directed research is based on detailed review of research supported by both the NINCDS extramural program and other federal agencies and on suggestions from ad hoc advisors and the Collaborative and Field Research Advisory Committee. The program concentrates attention on areas of pressing research need and sponsors projects having exemplary research designs.

The five-year program plan for Communicative Disorders contains four major objectives and twelve program elements which are described briefly below.

Objective I: Improved Diagnosis, Treatment Amelioration, and Prevention of Disorders of the Auditory System.

The first program element under this objective concerns detection of hearing and other auditory disorders. A contractor has attempted to develop screening procedures for use by parents or paraprofessional personnel in determining whether or not they should seek a diagnostic workup of the child's hearing. Another contractor is developing a manual describing detailed procedures for administering impedance tests to very young children. The purpose of this manual will be dissemination of results of several research projects and of extensive clinical field trials.

Treatment of serous otitis media constitutes the second program element under this objective. It has been estimated that this condition affects approximately 20 percent of all children; clinical reports, however, provide no consensus concerning preferred treatment. A Request for Proposals to conduct a tightly controlled study of the effectiveness of several different treatment approaches for serous otitis media was advertised this year. Since no responding proposal was considered to be of adequate technical quality, no contract is being awarded this year. A Request for Proposals placing even greater emphasis on scientific aspects of the project may be advertised next year.

The third program element, presbycusis, is scheduled for initiation during FY 77. However, several of the projects currently underway and listed under the third objective, Aids for the Communicatively Handicapped, involve persons with presbycusis.

Objective II: Improved Detection, Assessment, Prevention and Treatment of Language and Speech Disorders Not Directly Attributable to Impaired Reception of Visual or Auditory Stimuli.

The first program element for which two contracts are expected to be awarded in June is improved assessment and differential diagnosis of children with language disabilities. One project will develop a composite of measures of children's language performance which will be capable of assessing small improvements which may occur during therapy among children with language disorders. The other project will conduct an experimental study of performance of a wide array of sensory, perceptual, and cognitive tasks among children whose speech or language problem is confined to a very specific disorder and who are without other problems. This project is expected to lead to improved diagnostic and classification procedures for children who have been referred to as "learning disabled" or "minimally brain damaged."

The second program element, treatment of language and speech disorders in children, will be fully initiated during FY 77. However, to provide a basis for program planning, a contract was awarded last year to an interdisciplinary team of scientists who are reviewing and synthesizing biological and behavioral evidence concerning the hypothesis of critical age(s) for language learning. The resulting document is expected to be published in the scientific literature.

The third program element, adult language disorders, was initiated last June with the award of a contract for developing a system for measuring and quantifying the communicative interactions between the speech pathologist and the aphasic adult during speech therapy. A new contract is expected to be awarded this spring to develop a measure of everyday communication skills of aphasic adults and to validate this measure as well as several of the existing aphasia tests applied to [illegible] of patients [illegible]. Both of these research tools must be developed before a definitive study of different treatments for aphasic adults may be initiated. Another intramural project is developing measures of the speech [illegible] to evaluate dysarthria in adults with neurologic disease. This project will also determine whether these objective measures are useful as indices of disease progression and for differential diagnosis of various types of dysarthria associated with neurologic disease.

Objective III. [illegible] for the Communicatively [illegible] Development of New Devices to Facilitate Language Reception and Expression by Persons with Disorders of Hearing, Speech, and Language.

The first program element within this objective is improved selective fitting of hearing aids. One contract project is continuing to develop prototype prescriptive procedures for fitting [illegible] to adult patients with acquired sensorineural hearing loss and also is developing [illegible] procedures to [illegible] reduced [illegible]. An intramural project is comparing [illegible] testing of persons having presbycusis [illegible] potential value of designing new types of hearing aids. Finally, a contract is expected to be awarded this spring for the [illegible] a dozen persons who are already fitted with currently available [illegible].

Two contractors have been engaged in projects concerning the second program element, tactile and visual aids for the deaf. One project has investigated speech reception with [illegible] visual [illegible], which are derived from computer analysis of the speech acoustic wave, supplement information available on the face of the talker. Another project has attempted to improve speech and vocalizations of very young profoundly [illegible] children by means of a tactile display [illegible]. [illegible] project is also investigating [illegible] electrical stimulation [illegible] hearing children and adults. The [illegible] will hold a brief, intensive workshop during the summer of 1975 with the following objectives: (1) Review of the current state of the art concerning visual and tactile aids to receptive communication, (2) identification of [illegible] and promising new areas, and (3) [illegible] and visual aids to facilitate communication [illegible] communication with hearing aids.

Objective IV. [illegible] Which Are Attributable to Noise Exposure.

The first program element [illegible] speech discrimination [illegible] for [illegible] a new type of [illegible] among [illegible].

The second element, concerned with [illegible] hearing of noise, is expected to be initiated in FY 1976.

The third program element, effects of noise on children, represents a major new research thrust which was initiated because very little information has been available. A review and analysis of the literature, which was commissioned a year ago and which will soon be published, enumerates a number of conclusions:

"1. Limited data from cats and guinea pigs exposed to high-level sounds suggest that young animals incur greater anatomical and physiological injuries of the inner ear than older animals. Very few data are available on noise-induced hearing loss in children. The qualitative and quantitative facts about the effects of age on noise-induced hearing loss and noise-induced injuries of the ear are thus essentially unknown.

2. A number of devices have sound levels which are capable of producing temporary, chronic, and permanent hearing losses in adults and probably children. Whether they do, however, depends upon the duration of exposure, number of exposures, and scheduling of exposures as well as the relative susceptibility of children.

3. Noise and some oto-toxic drugs have synergistic effects on the inner ears of experimental animals. Synergistic effects in young animals and humans have not been studied.

4. Retrospective studies suggest that habitual use of a powerful hearing aid probably does not produce permanent deterioration of hearing especially when the differences observed between aided and unaided ears are corrected for chronic, temporary threshold shifts of 5-20dB. In other instances where deterioration of hearing sensitivity is noted, it is often difficult to separate the hearing loss presumably caused by the hearing aid from progressive losses of hearing associated with genetic and other factors. The effects of habitual use of a powerful hearing aid on the hearing of infants and young children are not known.

5. Levels of noise which interfere minimally with the perception of speech by adults may interfere significantly with the perception of speech by children as well as with the acquisition of speech, language, and language related skills.

6. Although substantially more research is required, some results suggest that exposure to sufficient levels of urban noise for sufficiently lengthy periods or during critical development periods may have deleterious effects on auditory discrimination and possibly reading skills of children. It is not clear if these deficits in listening and in reading are temporary and will be overcome by maturation and schooling or whether these effects are permanent and not amenable to remedial measures.

7. Levels of short duration sounds which awaken adults from light sleep (Stage II) may not produce behavioral responses in sleeping infants. Levels of short-duration sounds that awaken adults from deep sleep (Stages III and IV) produce motor responses including startles in sleeping infants. Firm conclusions about the effects of noise on the sleep of infants and children are not possible until the biological significance of sleep patterns and their

agedependent changes will have predictive value.

3. Progress in evaluating the effects of noise in children required the development of suitable new instruments.

Three Requests for Proposals were submitted this year to address some of the important gaps in knowledge concerning effects of noise on children and the contracts are expected to be awarded in June. The first project will develop measuring techniques for assessing auditory sensitivity to pure tones and discrimination of complex environmental-like auditory signals in children under six years. The second phase of this work will examine the relations between performance on these measures and both biographical data and level of background noise during the tests. The second project will conduct a prospective, longitudinal study of young hearing impaired children who are fitted with their first hearing aids and will determine whether aids produce any additional hearing deficit, either temporary or permanent, in such children. The third new project will determine whether there are significant relations between children's noise environments and their auditory, speech, language, and reading skills when all variables other than noise exposure are controlled.

The fourth program planned, effects of noise on persons with ear diseases, was scheduled to have been issued during this fiscal year but the lack of a new staff member to assume responsibility for research on noise has led to postponement of this activity.

Coordination of Research on Noise. The head of the Communicative Disorders program has responsibility for coordinating all NINCDS research on noise with the other two HEW agencies concerned with related problems and, through the Environmental Protection Agency's Health Effects Panel, with all other government agencies concerned with the other effects of noise exposure. In cooperation with the National Institute of Environmental Health Sciences (NIEHS) and the National Institute of Occupational Safety and Health (NIOSH), NINCDS has submitted to the Division of Research Grants, NIH, a set of refined guidelines for assigning grant applications concerned with noise to one of the three agencies. When implemented, these guidelines will refer all research concerning occupational noise to NIOSH, all research concerning nonauditory environmental noise to NIEHS, and all research concerned with the auditory effects of noise to NINCDS.

Coordination and Other Research Activities. Program staff have served on the Panel on Early Childhood Research and Development, which was formed by the Office of Child Development in order to facilitate interagency research coordination. Overview contact has been maintained with the Task Force on Hearing Aids of the Food and Drug Administration and consultation has been provided to the staff member of that agency who is primarily concerned with developing standards for hearing aid manufacture. General consultation on many program matters has been provided to the American Speech and Hearing Association, the Bureau of Education for the Handicapped, and the Russell Sage Foundation. Finally, continuing technical assistance is provided to the NINCDS Office of Scientific and Health Reports.

One paper has been prepared for publication during this reporting period, the invited chapter, "Research on Normal Acquisition of Language," was written by Dr. Elliott for the NINCDS 25th anniversary volume. The paper mentioned in last year's report, "Temporal and Masking Phenomena in Persons with Sensorineural Hearing Loss," appeared in International Audiology, Volume 14(4), pp 336-353. Dr. Elliott also prepared an invited review of Hearing in Children by Northern and Downs for The Journal of the Acoustical Society of America.

Among the outside professional activities of members of the program, Dr. Elliott began a three-year term as a national officer of the Acoustical Society of America and also a term on the Publication Board of the American Speech and Hearing Association. Dr. Ludlow gave an invited talk, "The Recovery of Syntactic Structures of Aphasic Adults Following Stroke," to the New York City Aphasia Study Group. Finally, Dr. Cudahy gave a paper titled, "Fundamental Pitch Discrimination for Monotic and Dichotic Two-Tone Complexes," at the April meeting of the Acoustical Society of America.

Problems Encountered and Continuing Challenges: The major difficulty encountered has been insufficient staffing. In June, 1974 the Communicative Disorders program understood it was soon to become a branch with increased personnel but July plans for reorganization of the Institute caused postponement of branch status and increased staffing. There also have been changes in operating procedures which have placed additional demands on the limited staff and where advance work-scheduling was precluded.

Three features of directed research present continuing challenges. Most of the contracts awarded last year and many of those which are expected to be awarded this year, involve organizations which have not previously had contract experience. It is very encouraging to see these investigators become involved in contract activities but there are features of project management which apply to contracts and not to grants (where many of these scientists have had long experience). In addition to monitoring progress of the research itself, the Project Officers find they must devote extensive time to explaining general contract procedures. We hope to develop more efficient and effective procedures for enhancing field understanding of contract mechanisms and philosophies.

A second challenge concerns procedures for assisting offerors responding to Requests for Proposals to understand the scientific rigor required in controlled clinical research. Conversations have been held with representatives of several professional associations concerning possible short courses to be held at national meetings, but additional approaches would be useful and desirable. For this purpose, we are investigating texts and courses targeted for other fields which may be readily generalizeable to clinical research on communicative disorders. The preferred form for disseminating this information (e.g., annotated bibliography, commissioned journal articles, etc.) has not been selected.

The third continuing challenge concerns priority-setting for this directed research program. For the past two years, only projects which could be classified as pressing research needs have been undertaken. As the program

CONTRACT NARRATIVE
Communicative Disorders, C&FR, NINCDS
Fiscal Year 1975

JOHNS HOPKINS UNIVERSITY (N01-NS-2-2318)

Title: Study of Cutaneous and Visual Patterned Stimulation Communication Aids for the Profoundly Deaf Infant

Contractor's Project Director: Moise H. Goldstein, Ph.D.

Current Annual Level of Support: $49,959 (FY 74)

Objectives: The original objective of these studies was to develop techniques and instrumentation for studying and evaluating the feasibility of utilizing cutaneous or visual stimulation derived from a deaf infant's own vocalizations in order to provide information feedback and to increase the infant's vocalizations and speech. During the present contract year, an additional objective has been to obtain basic psychophysical information concerning tactile pattern discrimination in young children and adults.

Major Findings: Children in all three experimental groups (visual feedback, tactile feedback, and control group) showed increased vocalizations; substantial differences were not obtained among the three groups although more refined statistical analyses are continuing. Preliminary results of the psychophysical studies suggest that the cue of duration has particular salience for absolute identification of tactile stimuli.

Significance to NINCDS Program and Biomedical Research: The feasibility of tactile aids for the deaf deserves full exploration; this project will contribute useful information toward assessing suitability of tactile aids for very young children.

Cooperating Units: In previous years, this contract was monitored by the Biomedical Engineering Section.

Proposed Course of Contract: This contract will terminate in the early fall of 1975. The investigators may submit a grant application to continue specific aspects of the project. This summer, NINCDS is convening a workshop on Tactile and Visual Aids for the Deaf; two investigators from this contract will participate in that workshop and it is anticipated that workshop recommendations will suggest future directions for this type of research.

CONTRACT NARRATIVE
Communicative Disorders, C&FR, NINCDS
Fiscal Year 1975

BOLT BERANEK AND NEWMAN (N01-NS- *4-2322)*

Title: Development and Testing of an Instrument for Assessing Speech Discrimination

Contractor's Project Coordinator: Daniel N. Kalikow, Ph.D.

Current Annual Level of Support: $139,534 (FY 74; 18 months contract duration)

Objectives: This contract was awarded in June 1974 to develop a new test for measuring discrimination of connected speech where key words vary in predictability. This test is intended to measure the cognitive aspects of speech discrimination which are not assessed by presently available test instruments. In the second phase, beginning in late June, 1975, the contractor will measure speech discrimination in the presence of a competing speech message at various signal-to-noise levels for groups of subjects having normal hearing, conductive hearing-impairment, and sensorineural hearing impairment in order to assess reliability and predictive usefulness of the test in a controlled clinical setting.

Major Findings: A large pool of potential test items has been developed and administered to subjects. Selection of final lists of test items is underway and plans for clinical testing are being finalized.

Significance to NINCDS Program and Biomedical Research: Available clinical tests are not highly successful at identifying, among persons with acquired sensorineural deafness, those individuals who are likely to experience improved receptive communication with an aid. To the extent that successful aid wearers utilize redundancy and predictability of speech messages to improve their understanding of speech, this test will improve prediction of successful hearing aid use.

Proposed Course of Contract: Completing of Phase II is expected in December 1975. This should be followed by a more complete field evaluation of the new test under typical clinical circumstances.

CONTRACT NARRATIVE
Communicative Disorders, C&FR, NINCDS
Fiscal Year 1975

GALLAUDET COLLEGE (N01-NS-3-2316)

Title: A Study of Developing a Speech Analyzing Hearing Aid

Contractor's Project Coordinator: Dr. R. Orin Cornett, Ph.D.

Current Annual Level of Support: $223,180 (FY 73--2 years' funding)

Objectives: The contractor has been studying the feasibility of a speech analyzing aid to assist deaf individuals in lip reading by supplying supplemental cues derived from the acoustic speech waveform. The contract has two emphases: determining the capability of deaf subjects to integrate information from two different channels in real time and determining the feasibility of extracting the supplemental cues from the acoustic signal.

Major Findings: Deaf subjects are able to learn to use abstract cued aids to lip reading when the presentation rate is about half that of normal speech and is presented by keyboard-produced stimuli. (No data are presently available concerning real time rates of speech.) New cue groups have been established on the basis of these data. Computer-based speech analysis and derivation of cues has proven far more difficult than the contractor had anticipated.

Significance to NINCDS Program and Biomedical Research: It is well documented in the research literature that even under ideal conditions of good lighting, frontal view, and short distance from the speaker's face, deaf persons gain only a limited amount of information by lip reading. If one could develop a lip reading aid which supplements the visual face-and-mouth pattern with information concerning speech sounds not visible at the lips, the receptive communication skills of deaf persons would be measurably improved.

Cooperating Units: Prior to April 1, 1974 this contract was monitored by the Biomedical Engineering Section.

Proposed Course of Contract: The feasibility phase will terminate in June 1975 and there are no present plans to extend the contract during the summer of 1975. NINCDS will hold a planning, two-day workshop concerning visual and tactile aids for the deaf. It is anticipated that increased attention to the perceptual and cognitive principles which necessarily are involved in this type of receptive communication and to the difficulties of analyzing the acoustic speech waveform will lead to greater progress in future contracts.

CONTRACT NARRATIVE
Communicative Disorders, C&FR, NINCDS
Fiscal Year 1975

VETERANS ADMINISTRATION HOSPITAL, MINNEAPOLIS, MINNESOTA (NIH-NINCDS-74-09)

Title: Development of a Research Tool Concerning Speech and Language Therapy for Aphasic Adults

Contractor's Project Director: Robert H. Brookshire, Ph.D.

Current Annual Level of Support: $87,900 (FY 74)

Objectives: This project is developing a descriptive and quantifiable system for documenting the therapeutic process of speech and language rehabilitation of aphasic adults. When developed, the system will be used in studies of the comparative efficacy of different treatment approaches.

Major Findings: An on-line system has been developed which enables the observer to choose one alternative within each of a number of categories representing various aspects of clinician behavior and of patient response. Data are presently being collected for conducting "item analyses" of category alternatives and for measuring validity of the procedures.

Significance to NINCDS Program and Biomedical Research: This new procedure will provide a needed research tool. It will be used both in the evaluation of overall effectiveness of different therapy approaches and to examine the effectiveness of specific therapy components.

Proposed Course of Contract: The development and validation phase should be completed by June, 1975. In the second contract year a manual for training observers will be developed and evaluated. The contract is expected to be terminated in June, 1976.

CONTRACT NARRATIVE
Communicative Disorders, C&EP, NINCDS
Fiscal Year 1975

THE UNIVERSITY OF UTAH (N01-NS-4-2305)

Title: Development of a Manual for Impedance Testing in Children

Contractor's Project Director: Geary McCandless, Ph.D.

Current Annual Level of Support: $10,849 (FY 74)

Objectives: This manual will capture a large amount of practical experience of the principal investigator and consultants in applying impedance measurement procedures to young children. The manual is being written to enable audiologists who did not receive extensive training in impedance procedures during their clinical training (widespread use of these techniques is relatively new) to develop expertise in the practical aspects of testing children. Research results and new procedures developed under several NINCDS grants will also be included.

Significance to NINCDS Program and Biomedical Research: This is a dissemination activity and will contribute to delivery of health services for children. The impedance test procedure is especially useful for detecting serous otitis media, a condition which usually produces a conductive hearing impairment while the condition persists. These test procedures, which can be quickly administered and which require no cooperation on the child's part other than sitting reasonably still and refraining from talking or swallowing, can be utilized for large-scale screening activities in public schools, public health facilities, etc.

Proposed Course of Contract: The manual should be completed during the summer of 1975. A field evaluation of the manual is intended, but plans for this cannot be formulated until the completed draft version of the manual has been received.

CONTRACT NARRATIVE
Communicative Disorders, CDPB, NINCDS
Fiscal Year 1975

CITY UNIVERSITY OF NEW YORK GRADUATE CENTER (NO1-NS-4-2323)

Title: Prescriptive Fitting of Wearable Master Hearing Aids

Contract Project Director: Richard E.C. White

Co-Principal Investigator: Harry Levitt

Current Annual Level of Support: $198,1[illegible]6 (FY 74; 24 months funding)

Objectives: The goal of this project is to determine the degree to which wearable master hearing aids used as prescriptive laboratory devices or as modifiable training devices can enhance the final fitting of hearing aids and improve the communicative efficiency of the wearer. The special target population for this contract is middle aged persons with acquired sensorineural hearing loss who have not received benefit from conventional hearing aids.

Major Findings: The contractor has completed extensive calibration measurements on the wearable hearing aid units which were developed under a previous contract. Measured data recorded when the aid is positioned in or a mannikin do not completely correspond to measurements obtained in the typical test environment and this effect is being further explored. New test materials have been developed, experiments with hearing-impaired subjects are underway and the protocol for the second test phase of the contract should be established by September, 1975.

Significance to NINCDS Program and Biomedical Research: Many hearing-impaired persons complain that hearing aids do not improve their hearing. This contract will develop procedures for prescribing for individual patients the most successful ensemble of acoustic characteristics available within the capabilities of present hearing aid design.

Cooperating Units: The wearable master hearing aids were developed under contract to the Biomedical Engineering program, CDPB.

Proposed Course of Contract: The draft prescriptive protocol, which is being developed during Phase I, will be tested on a larger group of subjects having sensorineural hearing impairment during Phase II. The contract is expected to be completed within 24 months but may require an extension in time, without additional funds, for preparation of reports, etc. Upon successful conclusion of the project, consideration will be given to modifying the prescriptive protocol to fit the particular needs of another special patient group, such as geriatric patients or young patients with congenital but moderate hearing impairments. In addition, if results warrant, tape-recorded prescriptive aids will be developed so that the adaptive, prescriptive approach might be widely utilized without the need for clinical dependence on the somewhat fragile wearable laboratory devices.

CONTRACT NARRATIVE
Communicative Disorders, C&FR, NINCDS
Fiscal Year 1975

MASSACHUSETTS MENTAL HEALTH RESEARCH CORPORATION (N01-NS-4-2324)

Title: Critical Age for Language Learning

Contractor's Project Director: Simeon Locke, M.D.

Current Annual Level of Support: $83,419 (FY 74; 18 month funding)

Objectives: The contractor is expected to complete a critical review, analysis, and synthesis of currently existing biological and behavioral data pertaining to the hypothesis of a critical age or ages for human language learning. This critical review should be suitable for publication as a monograph or in a scientific journal. The contractor is also outlining research needed to achieve greater understanding of the process of human language learning and associated disorders as they relate to the critical age hypothesis.

Major Findings: The contractor is using a seminar approach to meeting these objectives. Contract outcomes cannot be assessed until completion of the documents.

Significance to NINCDS Program and Biomedical Research: There are large gaps in our understanding of the neurological bases of language and, as a result, there is limited understanding of many disorders of language development. Although behavioral scientists have occasionally shown interest in neurophysiological research, the reverse has infrequently occurred. These synthesizing documents are expected to stimulate interdisciplinary research on language development and to focus attention on research problems deserving special attention. After publication of the documents, NINCDS expects to receive an increased number of high quality, investigator-generated grant proposals on topics in this general area.

Cooperating Units: A pediatric neurologist from the Perinatal Research Branch, NINCDS, participated in a site visit to this contract in January, 1975 and has read and commented on some of the materials submitted by the contractor.

Proposed Course of Contract: Contract will terminate, as planned, at the end of December, 1975.

CONTRACT NARRATIVE
Communicative Disorders, C&FR, NINCDS
Fiscal Year 1975

COMMITTEE ON HEARING, BIOACOUSTICS, AND BIOMECHANICS, NATIONAL RESEARCH COUNCIL (2Y01 NS 5001-02)

Title: Effects of Noise on Children

Current Annual Level of Support. $20,000 (FY 74)

Objectives: Since very little research has been conducted concerning effects of noise on children, a critical review and synthesis of the available information was commissioned in the spring of 1974 to serve as a basis for program planning in this general area.

Major Findings: Conclusions of the report are:

1. Limited data from cats and guinea pigs exposed to high-level sounds suggest that young animals incur greater anatomical and physiological injuries of the inner ear than older animals. Very few data are available on noise-induced hearing loss in children. The qualitative and quantitative facts about the effects of age on noise-induced hearing loss and noise-induced injuries of the ear are thus essentially unknown.

2. A number of devices have sound levels which are capable of producing temporary, chronic, and permanent hearing losses in adults and probably children. Whether they do, however, depends upon the duration of exposure, number of exposures, and scheduling of exposures as well as the relative susceptibility of children.

3. Noise and some oto-toxic drugs have synergistic effects on the inner ears of experimental animals. Synergistic effects in young animals and humans have not been studied.

4. Retrospective studies suggest that habitual use of a powerful hearing aid probably does not produce permanent deterioration of hearing especially when the differences observed between aided and unaided ears are corrected for chronic, temporary threshold shifts of 5-20dB. In other instances where deterioration of hearing sensitivity is noted, it is often difficult to separate the hearing loss presumably caused by the hearing aid from progressive losses of hearing associated with genetic and other factors. The effects of habitual use of a powerful hearing aid on the hearing of infants and young children are not known.

5. Levels of noise which interfere minimally with the perception of speech by adults may interfere significantly with the perception of speech by children as well as with the acquisition of speech, language, and language related skills.

6. Although substantially more research is required, some results suggest that exposure to sufficient levels of urban noise for sufficiently lengthy periods or during critical development periods may have deleterious effects on auditory discrimination and possibly reading skills of children. It is not clear if these deficits in listening and in reading are temporary and will be overcome by maturation and schooling or whether these effects are permanent and not amenable to remedial measures.

7. Levels of short duration sounds which awaken adults from light sleep (Stage II) may not produce behavioral responses in sleeping infants. Levels of short-duration sounds that awaken adults from deep sleep (Stages III and IV) produce motor responses including startles in sleeping infants. Firm conclusions about the effects of noise on the sleep of infants and children are not possible until the biological significance of sleep patterns and their age-dependent characteristics are understood better.

8. Progress in evaluating the effects of noise in children requires the development of suitable test instruments.

Proposed Course of Contract: The review document has been completed and is expected to be published in several months. The contract is being extended in time to allow for payment of publication page charges. Three Requests for Proposals concerning effects of noise on children have been advertised and the resulting contracts are expected to be awarded in the next several weeks. The titles of the three RFP's are: Auditory Sensitivity and Discrimination in Very Young Children; Possible Effects of Hearing Aids on Auditory Sensitivity; and Possible Relations Between Children's Noise Exposure and Auditory, Speech Language and Reading Skills.

Project No. Z01 NS 02102-02 C&FR
1. Office of Associate Director
2. Communicative Disorders
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Temporal and Masking Phenomena in Persons with Sensorineural Hearing Loss

Previous Serial Number: NDS(CF)-74 CD 2102-01

Principal Investigators: Lois L. Elliott, Ph.D.
Edward A. Cudahy, Ph.D.

Other Investigators: None

Cooperating Units: None

Person Years:

Total:	0.8
Professional:	0.8
Other:	0

Project Description:

Objectives: Persons with a hearing loss due to aging (presbycusis) or to noise exposure frequently complain of difficulty in understanding speech in a noisy environment. Previous investigations by Elliott and others have indicated that persons with sensorineural hearing loss have abnormalities in the temporal processing of acoustic signals in addition to their inability to detect sounds at normal intensity levels. The purpose of this project is to examine the target population's perception of auditory signals when temporal and frequency parameters are systematically varied.

Methods Employed: In the temporal integration studies, ability to detect a tone in the presence of continuous broad-band noise is measured as a function of the duration and frequency of the tone. The forward and backward masking studies measure detection of a brief tonal signal presented at various time intervals relative to the onset or termination of a long duration broad-band noise masker. All subjects are tested under all conditions with a standard two-alternative forced-choice procedure.

Subjects: Adult volunteers with pure tone audiograms indicating presbycusis, noise-induced hearing loss, and normal hearing (control groups) are employed through the Normal Volunteer Program. Subjects are pledged to participate for three test sessions per week over a minimum period of six months.

Major Findings: Since the laboratory was not completed until late in the fiscal year data collection has only recently begun and results must be regarded as tentative. As noted in previous investigations, both presbycusic and noise-induced hearing loss subjects have less steep temporal integration functions than normal controls, indicating decreased integration of acoustic energy. Two points should be noted: (1) Subjects with high frequency sensorineural hearing loss have normal temporal integration functions at low frequencies where their hearing thresholds are normal. (2) The temporal integration functions of subjects with noise-induced hearing loss appear to reach asymptote at stimulus durations of 100-msec for frequencies of hearing loss as contrasted with asymptotes at 500-msec for normal and presbycusic subjects. Insufficient forward and backward masking data have been collected to permit tentative conclusions.

Significance to NINCDS and the Program of the Institute: The abnormal time vs. energy relations displayed by persons with sensorineural hearing loss and the apparent frequency-specific nature of this distortion further explicate this population's poor speech discrimination in noise. If further data support these preliminary findings, design of improved types of hearing aids would necessarily have to consider modification of temporal as well as of intensity parameters of the signal.

Proposed Course of Contract: Data collection will be completed for the temporal integration and the forward, backward, and gap masking studies. Narrow-band maskers, as opposed to the broad-band masker of the above mentioned studies, will be used to simulate a more speech-like masking situation. With these investigations, the focus of the project will broaden to include abnormalities in the processing of frequency information in the context of temporal processing demands.

Keyword Descriptors: auditory temporal masking
sensorineural hearing impairment
presbycusis

Honors and Awards: None

Publications: Elliott, L.L.: Temporal and Masking Phenomena in Persons with Sensorineural Hearing Loss. *Int. Audiol.* 14(4): 336-353, 1975.

Project No. Z01 NS 02185-01 C&FR
1. Office of Associate Director
2. Communicative Disorders
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Characteristics of Dysarthric Speech Associated with Neurologic Disease

Previous Serial Number: None

Principal Investigator: Christy L. Ludlow, Ph.D.

Other Investigators: Virginia Geoffrey, M.S.
Ronald Kartzinel, M.D.
Donald Calne, M.D.

Cooperating Units: Clinical Director
NINCDS Intramural Research

Person Years:

Total:	0.10
Professional:	0.10
Others:	0

Project Description:

Objectives: This project is developing measures of the speech acoustic waveform to evaluate dysarthria in adults with neurologic disease and to determine whether these objective measures can be used for the assessment of disease progression and for differential diagnosis among the various types of dysarthria associated with neurologic disease.

Methods Employed: Tape recordings are made of subjects performing speech tasks (such as extended phonation, loudness and pitch variation, pause and rate control and rapid initiation) in response to a uniform set of instructions. Objective measures of the speech of Parkinson patients are being contrasted with those of normal adults of the same age range. Effects of L-Dopa, Bromocriptine, and disease progression are measured in a double blind study lasting six months. Contrasting patterns of speech impairment are being sought among three groups: Parkinson patients with L-Dopa-induced dyskinesia, Parkinson patients with rigidity and tremor, and patients with Huntington's Chorea.

Subjects: Patients with Parkinson's disease are being examined under various conditions at two-week intervals over six months. In addition, patients with Huntington's Chorea and normal adult volunteers are being examined.

Major Findings: Instructions for the speech tasks have been recorded on a stimulus tape to insure identical conditions during all subject testing and retesting. Objective criteria have been defined for measuring several different aspects of speech production from spectrographic analyses of recorded speech.

Significance to NINCDS and Biomedical Research: The development of objective procedures for the assessment and differential diagnosis of various types of dysarthria in adults with neurological diseases will enable researchers and clinicians to evaluate and improve various types of treatments for adults with dysarthria.

Proposed Course of Contract: Subject testing was begun in March, 1975 and should be completed by September, 1975. Data processing and analysis phases are expected to continue until spring 1976.

Keyword Descriptors: dysarthria
Parkinson's disease
speech science

Honors and Awards: None

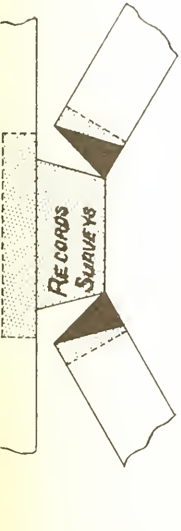
Publications: None

OW TO USE HESE SEPARATORS

se one page for ach separation.

elect appropriate ab, add further dentification if esired, and cover t with scotch ape.

ut off and discard ll tabs except the ne covered by tape.



TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

ANNUAL REPORT
July 1, 1974 through June 30, 1975
Section on Biomedical Engineering and Instrumentation
Collaborative and Field Research
National Institute of Neurological and
Communicative Disorders and Stroke
National Institutes of Health

Introduction

The Section on Biomedical Engineering and Instrumentation supports directed research projects for the purpose of developing instrumentation needed to advance the diagnosis and treatment of neurological and sensory disorders. The Section directs programs to adapt and apply recent advances in technology to problems in identified areas of neurosciences for which biomedical instrumentation is needed. The Section coordinates projects with other laboratories and branches of the Institute and the other biomedical engineering activities of the NIH. By working directly with the clinicians and scientists on their instrumentation problems, the Section seeks to provide the necessary interdisciplinary interface between the clinical problems and the technical development in order to assure the proper coordination of technical and clinical input at all stages of development.

The Section develops prototype instrumentation to adapt feasible approaches and techniques into practical clinical instruments. The Section monitors the clinical testing and evaluation of prototype instrumentation and provides for needed modifications and refinements to lead to useful practical clinical tools for commercial development and wide clinical application. The Section has supported several directed research contracts for feasibility studies and instrumentation aimed at the development of clinical instrumentation to meet high priority needs in fields related to the mission of the NINCDS. These include the following contracts:

NO1-NS-2-2323 - Ohio State University Research Foundation
Semiconductor Gamma Camera Development

NO1-NS-3-2319 - Indianapolis Center for Advanced Research
"Study and Test Ultrasonic Techniques for
Diagnosis of Cerebral Disorders

NO1-NS-4-2304 - Bowman Gray School of Medicine
"Study and Test Ultrasound Techniques for
Diagnosis of Cerebral Disorders"

In addition, the Section has assisted the Applied Neurologic Research Branch by monitoring the following contract funded by their program area:

NO1-NS-3-2322 - Stanford Research Institute
"Wearable EEG Tape Recorder"

Through an interagency agreement with the National Science Foundation, the Section has partially supported the preparation of a state-of-the-art report on Imaging in Radiology and Nuclear Medicine.

CONTRACT NARRATIVE
Biomedical Engineering and Instrumentation Section, C&FR, NINCDS
July 1, 1974 - June 30, 1975

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (N01-NS-2-2323)

Title: To Develop a Semiconductor Gamma Camera System for Nuclear Medicine

Contractor's Project Director: Robert F. Redmond, Ph.D.

Current Annual Level of Support: $36,871 (6 months)

Objectives: This research program has been directed to the development of a prototype semiconductor gamma ray camera for nuclear medicine, the results of which have led to a proposed design for a practical clinical camera employing high purity, strip electrode germanium detectors.

Major Findings: The principal accomplishments have been: 1) the development of techniques to fabricate orthogonal strip, high purity germanium detectors, 2) the development of an analytical model of the detector-charge dividing resistor network with associated filtering electronics, 3) the design and construction of an electronic system and to optimize the signal to noise ratio of the system and to display the images from the prototype camera, 4) the design and construction of high resolution, low cost collimators, and 5) the design of a clinical germanium gamma ray camera.

The proposed clinical camera will have an approximately 36-square-inch field of review and is projected to exhibit an energy and spatial resolution of 3.5 keV and 3.8 mm, respectively. The camera detector element will be assembled from sixteen 3.8 cm x 3.8 cm x 2 cm strip electrode detectors electrically connected in quadrants measuring 7.6 cm x 7.6 cm.

Significance to NINCDS Program and Biomedical Research: It has been shown through an experimental development and design program that germanium cameras can become a reality in nuclear medicine. The introduction of semiconductor cameras in nuclear medicine in turn may lead to significant improvements in medical diagnostic capability through improved image quality and spatial resolutions, and through the implementation of multi-isotope studies.

Proposed Course of Contract: Work under this contract was completed at the end of February 1975. It appears at this time that it would be most advantageous to use the small prototype for biological investigation. If it can be definitively demonstrated that the device has potential for unique clinical applications, construction of a scaled-up clinical version of the instrument may be considered.

CONTRACT NARRATIVE
Biomedical Engineering and Instrumentation Section, C&FR, NINCDS
July 1, 1974 - June 30, 1975

INDIANAPOLIS CENTER FOR ADVANCED RESEARCH (ICFAR) (N01-NS-3-2319)

Title: Study and Test Ultrasonic Techniques for Diagnosis of Cerebral Disorders

Contractor's Project Director: Francis J. Fry

Current Annual Level of Funding: $285,163 (28 months)

Objectives: This project, coordinated by and primarily performed at the Indianapolis Center for Advanced Research (ICFAR) with subcontract agreements to Bolt, Beranek and Newman (BBN) and Purdue University, is studying the feasibility of processing ultrasound return signals to acoustically identify and characterize the status of intracranial structures, and is evaluating the potential of these techniques for identifying specific intracranial lesions. In addition, prototype instrumentation and on-line computer processing are being developed to support trials of these techniques in a laboratory environment. These instrumentation development efforts are supplemented by studies of the acoustic properties of normal and pathologic brain tissue to be used for interpretation of the measured acoustic characteristics of intracranial structures for the ultimate purpose of non-invasive diagnosis of lesions and serial tracking of recovery following treatment.

Major Findings: This period was spent in describing the nature of the problem of interrogating intracranial contents through the intact skull from the perspective of the acoustical properties of the tissues involved, making preliminary measurements of acoustical properties of the excised skull and physical targets, and implementing on the facilities of the Fortune-Fry Research Laboratories of the Indianapolis Center for Advanced Research (FFRL-ICFAR) several algorithms operating in the time domain which are intended to yield an acoustic impedance profile on physical model systems. These model systems are ultimately to simulate the situation involving the intact human head (scalp, skull and intracranial contents) so that any potential clinical application, device or method will have had a relevant evaluation. Data from the literature and other sources have been compiled to help define the nature of the medical problem with respect to the acoustic parameters of skull and brain soft tissues.

Significance to NINCDS Programs and Biomedical Research: The diagnosis and treatment of stroke, head injury, brain tumor, and other intracranial disorders is of prime interest to NINCDS and the scientific fields that it represents. It is felt that non-invasive techniques, both for screening for cerebral disorders and for diagnosis of specific pathologies and their serial assessment following treatment, would be of great use in improving the clinician's capabilities in diagnosis and treatment of these disorders. If successful, the techniques being investigated under this contract will provide an extended capability for such non-invasive assessment of brain tissue status which should be of value for such diagnosis and serial assessment.

Proposed Course of Contract: The next phase of the experimental work will involve: 1) attempts to demonstrate the feasibility of obtaining impedance profiles of live animal brain (with the skull removed) with specific experimental infarcts; and, 2) attempts to describe quantitatively the effects of cancellus bone (preferably live) on the ultrasonic interrogating waveform.

CONTRACT NARRATIVE
Biomedical Engineering and Instrumentation Section, C&FR, NINCDS
July 1, 1974 - June 30, 1975

BOWMAN GRAY SCHOOL OF MEDICINE (N01-NS-4-2304)

Title: Study and Test of Ultrasound Techniques for Diagnosis of Cerebral Disorders

Contractor's Project Director: Ralph W. Barnes, Ph.D.

Current Annual Level of Support: $169,274 (24 months)

Objectives: These studies will measure and characterize the acoustic properties of normal and pathologic intracranial tissues to gather a data base with which to study the identification of specific pathologic states based on analysis of signal return from ultrasound interrogation. In addition, the contractor will develop and clinically evaluate techniques and instrumentation for non-invasive assessment of cerebrovascular dynamics through the use of the moving target indicator approach. These studies will be coordinated with those of ICFAR (see contract #N01-NS-3-2319) and other future contractors monitored by NINCDS.

Major Findings: Progress on this project can be divided into three areas, 1) computer programming, 2) acoustical measurements, and 3) detection of intracranial vasculature echoes. In the first area of computer programming, programs have been written, debugged, and modified where necessary for the frequency analysis of echoes. In the second area of acoustical measurements, all equipment and laboratory facilities have been assembled, and experiments will start shortly. In the third area of intracranial vasculature echo detection, optimal digital moving target indicator system parameters have been determined for the vasculature from the carotid system in the neck and smaller arteries about 2-3mm in diameter in the head. This laboratory information is proving to be valuable in the evaluation of patients.

Significance to NINCDS Programs and Biomedical Research: The diagnosis and treatment of stroke and other intracranial disorders is a major concern to NINCDS and to the medical fields related to the Institute's mission. The non-invasive diagnostic techniques to be investigated under this project will, if successful, provide valuable tools for non-invasive diagnosis of major forms of cerebrovascular disease, as well as providing additional capabilities for diagnostic interpretation of ultrasound interrogation of other tissues and structures within the cranial cavity.

Proposed Course of Contract: Future plans include frequency analysis of returned echoes from tissue samples; measurement of acoustical parameters of velocity, scattering, and absorption in tissue; imaging of intracranial vessels; and, measurement of pressure pulse arrival time relative to the R-wave of the ECG at selected regions of the brain.

CONTRACT NARRATIVE
Biomedical Engineering and Instrumentation Section, C&FR, NINCDS
July 1, 1974 - June 30, 1975

STANFORD RESEARCH INSTITUTE (SRI) (N01-NS-3-2322)

Title: Development of a Wearable Four-Channel EEG Cassette Recording System

Contractor's Project Director: Joseph H. Chadwick, Ph.D.

Current Annual Level of Funding: $101,800

Objectives: The objective of this effort is to design, fabricate, and clinically test prototypes of wearable EEG recording systems and associated play back systems which will make it possible to record, from ambulatory patients, four channels of EEG during a twelve-hour period using commercially available magnetic tape cassettes and play these back at high speed for computer analysis and clinical evaluation. This development is to include electrodes and preamps to provide for four channels of differential recording connected to a wearable tape recorder with appropriate signal processing electronics and digital encoding circuitry to provide for a full dynamic range and suitable bandwidth to allow for automatic processing of EEG data by computer. In addition to the design and development of the recording technique and implementation in hardware, the overall system is to be clinically evaluated with a selected group of patients under environmental conditions similar to those for which it will ultimately be used. The playback system is to transcribe the encoded data from initial tape cassettes and reform that data onto conventional computer compatible digital tape, as well as to interface directly to a computer and provide real time analog outputs for strip chart recording.

Major Findings: A prototype system was delivered in September 1974. Four leads of EEG waveforms can be recorded for twelve hours on a standard C-120 audio cassette. The bandwidth for each lead is 40Hz. The weight of the electronics and batteries in the recorder that has been delivered is approximately four pounds, and the weight of the vest is approximately one pound. A study has been conducted of methods for decreasing the weight of the recorder and increasing the system's capacity and reliability. The study included a clinical and life test, studies of new lightweight recorder design and a variable-speed recorder transport, and studies of methods for increasing the recorder capacity by using data compression.

Proposed Course of the Contract: The design goal for the second generation on-body system is three pounds. There appear to be no technical reasons why the recorder capacity cannot be increased to eight channels for twelve hours. The possibility of using a small minicomputer to perform most of the decoder-logic function will be investigated. Error correction will be incorporated into the playback units. It is believed that the additional circuitry required for the on-body subsystem when data compression is used will be simple and lightweight.

Collaborating Units: This project is being carried out under combined collaborative effort of the Applied Neurologic Research Branch and the Section on Biomedical Engineering and Instrumentation. The Applied Neurologic Research Branch is providing the clinical direction and the funding, while the Biomedical Engineering Section is providing the technical direction and project management.

Serial No. Z01 NS 02047-02 C&FR
1. Office of Associate Director
2. Section on Biomedical Engineering and Instrumentation
3. Bethesda, MD

PHS-NIH
Individual Project Report
July 1, 1975 through June 30, 1975

roject Title: Biomedical Engineering and Instrumentation Studies

revious Serial Number: NDS(CF)-73 BEI 2047

rincipal Investigator:

ther Investigators:

ooperating Units:

an Years:

Total:	0.0
Professional:	0.0
Others:	0.0

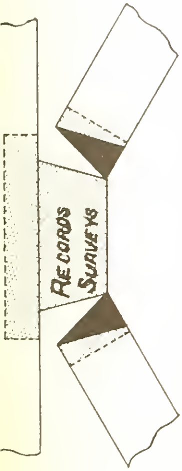
roject Description: This project has been discontinued due to the transfer of the Principal Investigator to another Institute.

)W TO USE
HESE SEPARATORS

se one page for
ach separation.

elect appropriate
ab, add further
dentification if
esired, and cover
t with scotch
ape.

ut off and discard
ll tabs except the
e covered by tape.



TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

ANNUAL REPORT
July 1, 1974--June 30, 1975

Applied Neurologic Research Branch
Collaborative and Field Research
National Institute of Neurological and Communicative Disorders and Stroke
National Institutes of Health

The Institute's Collaborative and Field Research programs in Epilepsy, Head Injury, and Stroke were conducted by the Applied Neurologic Research Branch. In-house research, directed (contract) research, and information programs were aided by support from the NIH Epilepsy Advisory Committee and its Subcommittees; an ad hoc advisory group provides advice on head injury and stroke.

Section on Epilepsy

Due to a requirement for additional laboratory space in Building 36, NINCDS relocated the Section on Epilepsy, Applied Neurologic Research Branch, to the first floor of the Federal Building at 7550 Wisconsin Avenue. All of the Section's activities--except for the pharmacology laboratory which remained in Building 36--were affected. The television and electronics laboratory and the computer laboratory were relocated. A move of this magnitude, which was planned and postponed innumerable times, was very unsettling to the personnel and conduct of ongoing programs. However, the staff is now situated and the only major remaining problems are the arrival and installation of new equipment, and the satisfactory resolution of environmental factors to permit the operation of video and telemetry equipment. As contrasted with buildings on the NIH reservation, the Federal Building does not provide around the clock environmental control, and video and audio tapes are affected by heat and humidity fluctuation. A resolution to this problem is being sought by GSA and NIH. A positive aspect of the relocation is that the epilepsy pharmacology laboratory in Building 36 will be expanded to include a gas-chromatography mass-spectrometry computer system. This will provide wide research opportunities as well as service our anticonvulsant drug developmental program. Another positive aspect of the relocation is the acquisition of a PDP 11/40 minicomputer in the Federal Building. This equipment will be used for data storage and retrieval in the posttraumatic epilepsy study, absence drug studies, and anticonvulsant drug development program.

Last year two drugs in which NINCDS controlled clinical trials were involved approached the market with the primary indication of seizure control. These two drugs are the first primary antiepileptics marketed in the United States since 1960. One of these, carbamazepine (Tegretol) was marketed in August, 1974, for this indication. The other, clonazepam (Clonopin) was marketed in June, 1975. Clonazepam was introduced for treatment of absence seizures which have not responded to treatment with ethosuximide, and other nongeneralized seizures.

There has been international recognition of the Section on Epilepsy for leadership in research and application. Numerous national and international lectures on epilepsy and antiepileptic drugs were given by invitation this year. There have been also visits from U.S. and overseas scientists for exchange of information on topics of mutual interest. J. Kiffin Penry, M.D., is serving as President of the American Epilepsy Society, and as Secretary General of the International League Against Epilepsy. These offices require much time but are extremely valuable in augmenting a leadership role in epilepsy for the Institute. Many Insitute-initiated programs are thus carried forward on a national and international basis.

NINCDS funded 11 studies to determine the feasibility of successfully establishing a comprehensive epilepsy program. Each of the 11 contractors is investigating this problem in a selected geographic area. It is anticipated that in many locations, a comprehensive epilepsy program will be possible to broaden clinical research in epilepsy in an appropriate setting, and at the same time assure that the newest findings are made rapidly available to patients.

During the year, the Institute sought proposals for the conduct of comprehensive epilepsy programs. Proposals were not limited to the 11 feasibility studies, but were sought from other potential contractors who could show evidence for the feasibility of establishing a program in their own geographic area. The goal of the conduct of comprehensive epilepsy programs is to facilitate applied research and to coordinate research and teaching with health care services related to persons with all types of epileptic seizures within a defined geographic area. Each program will be required to have inpatient facilities available to patients when required for periods of up to 3 to 6 months to receive special diagnostic evaluation and intensive treatment of their seizure disorder, and any other handicapping or physical problem. Vocational training, psychological support, and other ancillary services and continued schooling should be also provided. Existing services to persons with epilepsy should be coordinated by the Program for maximum utilization--this is a key feature of the conduct of comprehensive programs. An integral part of the program will be the demonstration at all levels in the management of patients with epilepsy for professionals, paraprofessionals and the lay public. Basic research in the neurosciences would not be supported by this program, but it is considered desirable from an overall standpoint. The Institute will award one or more contracts for this program this year.

The second new program begun this year is the initial pharmacologic evaluation of potential anticonvulsant drugs. After considerable discussion and guidance from the Subcommittee on Anticonvulsant Drugs, the program was officially begun on January 1, 1975, when a research contract was awarded through competition to the University of Utah for the screening of approximately 1500 compounds during the first 18 months of the contract. Compounds will be received from academic and industrial sources at NINCDS, recorded and a portion sent to the University of Utah for screening. Data on these compounds is returned to the Institute where chemical and biologic data are integrated in the Section on Epilepsy minicomputer. A data management system was devised after consultation with officials of the National Cancer Institute, the Division of Computer Research and Technology, and the pharmaceutical industry. A brochure to announce the program was developed and sent to medicinal chemists of the pharmaceutical industry and in universities; a contractural agreement for industrial participation was also developed. In December, 1974, 700 copies of the announcement were mailed, and compounds were received very soon thereafter. Continual solicitation will be required to assure an adequate flow of compounds. In the eighteen months of the contract, The University of Utah developed procedures for anticonvulsant compounds, and standardized a number of known drugs for comparative data. An arrangement has been made with Chemical Abstracts Service to provide computerized chemical structures for compounds submitted to this program. This information will also be entered into the computer with biologic data from the University of Utah.

Concurrently, a pilot clinical research project concerned with the study of absence seizures has been conducted in the Clinical Center of the National Institutes of Health. Video recording and telemetered EEGs are employed to document and quantify the features of this seizure type. More recently, the Section on Epilepsy has been requested to establish a small, selected seizure unit on a pilot basis in NIH's Clinical Center. This service would be concerned with evaluation and close monitoring of patients with intractable seizures and also provide the opportunity for pilot anticonvulsant therapeutic studies. To a large extent, the establishment of this service is due to numerous requests received from physicians who have been unable to control seizures in very difficult circumstances.

The importance of monitoring blood concentrations of antiepileptic drugs has been demonstrated many times by the Section on Epilepsy, and it is increasingly being used by private laboratories and hospitals throughout the country. The efficacy and safety of drug treatment in epilepsy has been increased through the monitoring of blood concentrations. Thus, this procedure has allowed individualization of dose regimens, reveals ineffective drug intake and presence of interacting agents in individual patients on multiple-drug therapy. In the review, "Usefulness of blood levels of antiepileptic drugs" (Kutt, H., and Penry, J.K., Arch Neurol., 31, 283-288, 1974), served to document the benefits and limitations of this laboratory service. A survey made by the Epilepsy Foundation of America with collaboration from the Section on Epilepsy revealed unfortunately the lack of accuracy and uniformity among commercial laboratories to provide this service. The results of this survey will be published. A voluntary system of analysis of samples from cooperating laboratories is being piloted, as will the development of reference standards of antiepileptic drugs in a biologic matrix now under development through an interagency agreement between NINDS and the National Bureau of Standards.

Infantile spasms is a form of seizures which afflicts a significant number of children each year. There is a considerable lack of information about the cause of this type of seizures, and no satisfactory treatment. In view of the lack of information about this seizure type, a workshop on infantile spasms was held on May 3, 1974, in response to a suggestion from the Subcommittee on Anticonvulsant Drugs. All available information on this syndrome was gathered and reviewed in detail by Section on Epilepsy staff to form a basis for the workshop where new thoughts on the etiology of infantile spasms, and designs for clinical drug trials were discussed.

A book, Neurosurgical Management of the Epilepsies, (Advances in Neurology, Vol. 8, Raven Press), was published in January, 1975, as result of an international Workshop on Neurosurgery. Substantial progress has been made in understanding the pathology of convulsive disorders; a theoretical basis for surgical therapy may now be better understood. Recent advances in neurosurgery dictate a reevaluation of the criteria for selection of patients for surgical treatment.

Significant advances in surgical techniques and postoperative management are also discussed. Publication of the book--which has been very well received--followed a workshop held at NIH.

The Section on Epilepsy continues to be guided by the Subcommittee on Anticonvulsant Drugs of the NIH Epilepsy Advisory Committee. Two meeting were held; one in Seattle in September, 1974, and the other in Bethesda, March, 1975. The Subcommittee reviewed the progress of NINCDS sponsored-research contracts and proposals for continued and additional studies. Recommendations were made for program plans to be undertaken in the coming year.

The NINCDS epilepsy program obtains information from multiple sources on investigational anticonvulsant drugs. During the year, a novel compound from Hungary was disclosed to the Institute; also at least two U.S. pharmaceutical companies are developing antiepileptic drugs. These companies are not now in this field, but are obviously concerned with the need for better therapeutic agents in the treatment of seizures.

Sodium dipropylacetate is perhaps the most interesting investigational drug at this time, because of its reported wide spectrum of activity and lack of serious side effects. The drug is marketed in 11 countries, and an investigational new drug notice was submitted by Abbott Laboratories in December, 1974, for the investigation of this drug in this country. The Institute will cooperate in obtaining controlled studies of this promising agent.

Section on Head Injury and Stroke

In FY 1973, the functional statement and official designation of the Section on Head Injury, ANRB, C&FR, NINCDS, were modified to broaden the perspective of that Section to include two additional disease categories, namely, brain trauma and stroke. The goals and objectives of biomedical research management in both disease states are similar including: a) prevention of incidence (primary prevention) through risk-factor screening and prophylactic intervention; b) prevention of disability (secondary prevention) or preservation of neurological function in each new incident of the disease; and, finally, c) restoration of function(tertiary prevention) in survivors of each incident through substitutive rehabilitative techniques and appliances, along with prevention of recurrences.

During fiscal year 1975, the program on risk-factor screening in the stroke prone was focused on the unique premonitory symptoms identified in retrospective studies as stroke antecedents, transient ischemic attacks (TIA's). The Collaborative Study of the Hospital Frequency and Character of TIA achieved its predicted goal in the accession of over 1300 TIA suspects identified at six participating university hospitals. Patients are currently completing the prescribed period of short-term follow-up. The overall study will be phased out and reported in a series of scientific publications during the first half of FY 1976. Data from the study will be used to develop formulas on the reliability of the diagnosis based on patient histories, the variability in the duration of individual TIA's and will provide a data base of proven feasibility for the planning of later studies on prophylactic intervention.

During FY 1975, under contract with Duke University, a self-administered questionnaire was developed and field tested for sensitivity and validity, which is designed to detect TIA's in the high-risk population of aging persons living in the community outside of hospitals. The questionnaire is now in serial use in five demographically selected cities to test the incidence and prevalence of TIA's prospectively in this high stroke-risk subsample of Americans in the retirement years.

The Section participated in the planning of a National Survey on Incidence, Prevalence and Cost to the Nation of Stroke to be initiated and conducted by the Office Biometry, OD, NINCDS. A similar Survey on Incidence, Prevalence and Cost of Head and Spinal Cord Injury was launched and got underway during the past year under contract with the Research Triangle Institute, Raleigh-Durham, North Carolina. When available, data from these utilization surveys will be useful in planning treatment units and research programs addressed to the objective of secondary prevention or "preservation of function."

Directly related to the latter objective, addressing the issues of duration and quality of survival, during FY 1975 the NINCDS Collaborative Study of Cerebral Survival (the brain death survey) was brought to a close. The final report of the program director was received by the Institute, editorially previewed, and several major scientific reports are now in press or in manuscript. These include reports on the pathology found in association with the brain death syndrome, the respirator brain, the summary report on the validity and accuracy of clinical and EEG findings, and a recommendation on the approach to a definition and legislation of death. In addition an Atlas of EEG Findings in Brain Death Suspects is planned and will be published as a monograph by certain participating investigators from the collaborative group.

Another project designed to refine the tools available for clinical assessment of acutely-ill accident and stroke victims became operational during 1975— the Study of Prognosis in Coma. This international collaborative project uses a digitized quantitative version of the Plum-Posner system of grading of stupor and coma, applied in the initial critical phases of stroke and brain trauma, in the attempt to predict outcome in terms of quality of recovery at six months. In addition to prognostic information, successful completion of this project will provide a field tested, validated and standardized quantitative instrument for the prospective assessment of comatose patients in intensive care units.

Specifically addressed to quality of survival after stroke, a study was undertaken at Howard University requiring longitudinal follow-up for six months after entry of acute stroke patients. The project is also a prospective test of clinical signs and symptoms reputed to have prognostic value in retrospective data analyses.

A key element in improvement of the state of the art in acute care is the development of noninvasive methods for early diagnosis, precise localization and quantitative measurement of central nervous system lesions. The instrumentation breakthrough spearheaded by EMI Corporation of England has obviated the priority previously set by the Section for development of a high resolution gamma camera. The project to develop a biplanar germanium sensor at Ohio State

University was phased out during FY 1975. A contract to field test the power of resolution in time and space of computer-assisted axial tomography (CAT scans) was awarded late in FY1974 to the Mayo Foundation, and work on the project began in 1975. This project will use CAT scans in the attempt to distinguish small infarcts or lacunes occurring in neurologically silent areas from TIA's and seeks to determine the latency over which ischemia alters the x-ray absorbency of cerebral tissue.

In FY 1975, a similar contract will be awarded after peer review and selection among offerers to identify and resolve logistical problems inherent in CAT scanning of trauma patients and to query the instrument's ability to distinguish and delineate the multifocal lesions of heterogeneous quality which occur in the severely traumatized brains of automotive accident victims. The sensitivity of CAT scans will also be tested in less severe cases in which the differential diagnosis lies between concussion and cerebral contusion. Together these projects will provide data which will identify both the utility and pitfalls of CAT scans which are destined to become integral parts of head injury and stroke comprehensive research programs.

Contract proposals are actively being sought in the current fiscal year to field test for accuracy, practicality, and potential for standardization of any one of several epidural pressure monitoring devices. Any of several approaches which have been recently proselytized and tested in animals would provide a major breakthrough in quantitative measurement of intracranial pressure (ICP) and thereby provide an objective tool for following the pathophysiology inherent in the natural history of brain trauma and certain strokes. Furthermore, reliable ICP monitoring would permit quantitative assessment of the effects of therapy in these types of cases.

Two projects have been in progress throughout FY 1975--one at New York University and the other at the University of Minnesota--to validate a previously reported technique for the qualitative detection of cerebral circulation. Intravenously injected radionuclide pertechnetate is reputed to produce a "bolus" effect in both the head and peripheral vascular beds externally monitored using sodium iodide crystal and radiation detection system. Brain death is defined in Europe in terms of total brain infarction and is therefore diagnosed whenever no circulation to the brain can be demonstrated by aortocranial angiography over a 3-minute period. This assurance, in addition to electrocortical silence, would be highly desirable in identification of potential organ donors and whenever cerebral viability is in question. The studies in progress attempt to resolve the question of reliability of the noninvasive pertechnetate method, using traditional transfemoral aortocranial angiography as the standard or control.

Tests of therapeutic efficacy of medical and surgical treatments designed to improve quality of recovery or reduce the size and distribution of the lesion came under intense study by the Section during 1975. Specifically, the protective effect of hypometabolic therapy (barbiturate drugs), the pejorative effects of certain anesthetic agents such as ketamine, and proposed therapies such as CO_2 inhalation are underway using superficial and deep probes; the pathogenesis, therapeutic and deleterious effects of cerebral ischemia upon cerebral blood flow and electrophysiological activity of the brain in the rhesus monkey are under study.

In 1975, planning and initiation of studies of controlled hypometabolic coma, surgical revascularization and embolectomy in experimental animals are in progress as a pretest of protocols proposed for clinical investigations in humans. None of these highly desirable modalities of great theoretical value in management of stroke and trauma have been adequately demonstrated as safe and efficacious in animals to justify application to humans. Nonetheless, they point the direction for future development of therapeutic breakthroughs for stroke and trauma victims if logistical problems, quantitative assessment tools, and monitoring devices to maintain patient safety can be resolved.

In the area of tertiary prevention or rehabilitation, the key to achievement of therapeutic goals lies in the higher nervous system functions of learning ability. Since once cerebral damage has occurred the patient must learn to substitute residual functions for those which have been lost, either with or without appliances, ability to learn these techniques and adapt to new and compromised circumstances is more important than the precise delineation of the lesion itself or its consequences. Since 1972, the Section has sponsored a project directed by Dr. Ralph Reitan on the recovery of neuropsychological functions after acute head injury. Forty-five (45) patients are in follow-up using serial psychometric batteries for up to two years. This study will be completed during the next fiscal year and is currently planned to include a complete CAT x-ray study on each patient for correlation of actual lesions with neuropsychological performance.

The program of the Section on Head Injury and Stroke, C&FP, NINCDS, has been and continues to be addressed to primary prevention, preservation of function and rehabilitation of these important disease categories. While considerable progress has been made in identification of factors and therapeutic modalities which determine survival, the major objections for future research in these areas are the determinants and favorable modification of factors which control the quality of survival.

CONTRACT NARRATIVE
Applied Neurologic Research Branch—Section on Epilepsy
July 1, 1976—June 30, 1977

NEW CASTLE STATE HOSPITAL (NO1-NS-[illegible])

Title: Development of a Model for Assessment of New Antiepileptic Agents

Contractor's Project Director: [illegible], M.D.

Current Annual Level: $[illegible]

Objectives: To further develop methods to study the efficacy, safety and availability of new antiepileptic drugs in humans. During the past year, the investigators initiated study of the anticonvulsant property of valproic acid as adjunct in the therapy of patients with generalized seizures refractory to treatment, the relationship of [illegible] and effects of the drug, and [illegible] role of drug serum concentrations.

Course of Contract: A pilot study of valproic acid efficacy and [illegible] drugs was completed during the summer of 1976. Additional data was collected and sent to the Section on Epilepsy, NINCDS, Bethesda, for review and prepared [illegible] for computer aided analysis. Analysis of this data is under way. During winter, 1976–spring, 1977, trials were done with valproic acid in [illegible] patients. A [illegible] protocol on [illegible] study was started in the spring of 1977.

Major Findings: Valproic acid appears to be an effective anticonvulsant as therapy of seizures resistant to treatment. Additional data on [illegible] usage of valproic acid is being analyzed. Continuation of this study will now determine the efficacy of valproic acid as an antiepileptic drug during longer time periods.

Significance to NINCDS Program and Biomedical Research: The pharmaceutical industry has demonstrated little interest in developing new antiepileptic agents. Aside from the economic factors involved, one of the industry's major problems is to obtain satisfactory clinical trials of new [illegible] drugs. Through this contract and others, NINCDS has supported clinical studies of antiepileptic drugs. With continued studies at facilities like New Castle State Hospital, there will be significant acceleration of development of new antiepileptic drugs and may encourage the pharmaceutical industry to develop promising agents for clinical trial. It is anticipated that well-designed studies will enable drugs to reach the market more rapidly, and thus be available to physicians who treat patients with epilepsy.

Proposed Course of Contract: Evaluation of other investigational antiepileptic drugs and further development of the model for drug evaluation are planned.

Publications:

Cereghino JJ, Brock JT, Van Meter JC, Penry JK, Smith LD, White BG. Carbamazepine for Epilepsy. A controlled prospective evaluation of multiple drug therapy. Clin Pharm Therap 16, 1975 (in press).

Cereghino JJ: Serum Carbamazepine Concentration and Clinical Control. In Penry JK and Daly DD (Eds.), Complex Partial Seizures and Their Treatment. Raven Press, 1975 (in press).

CONTRACT NARRATIVE
[illegible] Research Branch [illegible]
July 1, 1974–June 30, 1975

MEDICAL COLLEGE OF GEORGIA (NO1-NS-2-2335)

UNIVERSITY OF VIRGINIA [illegible] (NO1-NS-2-[illegible])

Title: Study of [illegible] Properties of Clonazepam [illegible]

[illegible]: [illegible]
[illegible]

Current Annual Level: $[illegible] Medical College of Georgia
$[illegible] University of Virginia [illegible]

Objectives: [illegible]

Status of Contract: [illegible]

Major Findings: [illegible] Clonazepam was effective in some patients with absence seizures who had not been previously treated. Also, clonazepam controlled some seizures which were refractory to ethosuximide. [illegible]

Proposed Course: Sodium dipropylacetate, a very promising antiepileptic [illegible] will be evaluated in this population. [illegible] and ethosuximide.

Publication:

Dreifuss, F.E., Penry, J.K., Rose, S.W., Kupferberg, H.J., Dyken, P., and Sato, S.: Serum clonazepam concentrations in children with absence seizures. Neurology 25:255-258, 1975.

[illegible]

[illegible]

[illegible]

[illegible]

Title: Study of Catheterization [illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

Publications

[illegible]

[illegible]

[illegible]

[illegible]

CONTRACT NARRATIVE
Applied Neurologic Research Branch--Section on Epilepsy
July 1, 1974--June 30, 1975

UNIVERSITY OF WASHINGTON (N01-NS-1-2282)

Title: Study of Experimental Antiepileptic Drugs in Animals

Contractor's Project Director: Joan S. Lockard, Ph.D.

Current Annual Level: $234,087

Objectives: To compare the antiepileptic efficacy of carbamazepine, phenobarbital, and diphenylhydantoin in spontaneous motor seizures of primates. Seizure frequency and behavioral toxicity are compared with drug dosage and drug blood concentration in the primate with spontaneous seizures.

Course of Contract: This animal model of focal motor epilepsy has been utilized to determine the propylactic efficacy of combined diphenylhydantoin/ phenobarbital treatment on posttraumatic epilepsy. Also, the basic kinetics of clonazepam, dipropylacetic acid, and ethosuximide were studied in the monkey. The behavioral precipitance of seizures in a group of free-roaming epileptic monkeys instrumented with motor seizure telemetry units was studied.

Major Findings: The half-life of clonazepam was determined to be 4-5 hours, and of dipropylacetic acid, 1/2 to 1 hour. Thus it was necessary to develop a system for catheterization whereby continuous administration of drugs and continuous monitoring of drug blood concentrations may be made. This system will be used to determine the efficacy of dipropylacetic acid, and clonazepam in this model. Six unrestrained animals with focal motor seizures were observed for six months using a fixed light/dark cycle in a free-room. Motor activity was used to telemeter EEGs; video recordings of seizures were made. This data forms a baseline for subsequent studies when drugs will be administered.

Proposed Course: The contract will be continued to further demonstrate and refine the usefulness of this animal model of epilepsy. The efficacy of dipropylacetic acid, clonazepam, clorazepate dipotassium, and ethosuximide are to be studied in the monkey model. The pharmacokinetics of dipropylacetic acid, clonazepam and ethosuximide are determined in the primate before efficacy experimentation is begun.

Publications:

Lockard JS: Motor seizure telemetry and initial data in free-roaming monkey model with spontaneous seizures. Epilepsia 16, 199, 1975.

Levy RH, Lockard JS, Green, JR, Friel P and Martis L: Pharmacokinetics of carbamazepine in monkeys following intravenous and oral administration. J Pharm Sci 64, 302-306, 1975.

Publications continued

Lockard JS, Congdon WC, DuCharme LL, and Huntsman BJ: Pharmacologic Propylaxis of Post-Traumatic Epilepsy in the Monkey Model. Soc. Neuroscience, 4th Annual Meeting Abstract, 1974.

Levy RH, and Martis L: Preliminary profile of dipropylacetic acid in monkey. Epilepsia 16, 202, 1975.

CONTRACT NARRATIVE
Applied Neurologic Research Branch--Section on Epilepsy
July 1, 1974--June 30, 1975

LAFAYETTE CLINIC (NO1-NS-2-2310)

Title: Evaluation of the Anticonvulsant and Psychologic Effects of Carbamazepine and Primidone in Outpatients with Temporal Lobe Epilepsy and/or Other Forms of Epilepsy.

Investigators: Ernst A. Rodin, M.D.
Philip Rennick, Ph.D.

Current Annual Level: $55,341

Objectives: To compare the antiepileptic efficacies of carbamazepine and primidone in blind cross-over fashion in outpatients with temporal lobe and/or other forms of epilepsy.

Proposed Course: The investigation has been completed, except for some comparisons to be made between 39 patients who had fewer seizures with primidone or with carbamazepine.

Major Findings: Forty-five patients completed the controlled comparison of primidone and carbamazepine; there were 17 dropouts. In the 45 patients, seizure frequency did not differ with the two drugs, and there were no severe side effects with either drug. However, there were more somatic complaints when the patients received carbamazepine. EEGs showed more slowing with carbamazepine; they showed fast activity when primidone was administered. Neuropsychological assessment showed less impairment with carbamazepine than primidone, due to the sedative properties of the latter.

Publication:

Rodin EA, Rim CS, and Rennick PM: The anticonvulsant effects of carbamazepine in patients with psychomotor epilepsy: Results of a double-blind study. Epilepsia 15:547-561, 1974.

CONTRACT NARRATIVE
Applied Neurologic Research Branch--Section on Epilepsy
July 1, 1974--June 30, 1975

UNIVERSITY OF KANSAS MEDICAL CENTER (NO1-NS-2-2313)

Title: Investigation of Posttraumatic Epilepsy Prophylaxis

Contractor's Project Director: Charles Brackett, M.D.

Current Annual Level: $57,000

Objectives: A pilot study to determine the effectiveness of propylactic treatment with diphenylhydantoin and phenobarbital in persons who suffer head injury and are thus liable to posttraumatic epilepsy.

Course of Contract: As of May 1, 1975, 114 patients have been accessioned in the study. Of this number, 27 patients have completed the required 18-month treatment period; all but one are continuing to be followed with neurological exams, plasma levels, and EEGs. There are 41 patients currently on drug therapy, and the study protocol is being scrupulously adhered to in their followup. Eight patients experienced seizures while on the study drug, and three have had seizures after completion of drug therapy. Data are being coded and stored in the PDP-11 mini-computer for analyses.

The admission rate is still less than anticipated due in part to the marked reduction in trauma admissions, although the study accessions are on a level with the previous year.

Proposed Course of Contract: Analyses to answer the proposed hypotheses will be done, and further investigation will be made into the relationship between the study drug dosage and plasma levels which are somewhat lower than expected. Determination will then be made for the requirement of additional studies.

CONTRACT NARRATIVE
Applied Neurologic Research Branch--Section on Epilepsy
July 1, 1974--June 30, 1975

EXCERPTA MEDICA FOUNDATION (NO1-NS-3-2303)

Title: Publication of Epilepsy Abstracts, Volume 8

Current Annual Level: $38,993

Objectives: To scan serial publications and periodicals from approximately 3000 of the world biomedical journals, select appropriate articles to be included in Epilepsy Abstracts in accordance with the guidance of the Project Officer and his editorial advisors; prepare abstracts with appropriate translations into English from foreign languages, classify, index, and store the abstracts in a computer retrievable form; and produce a 9-track computer tape for use at NIH. The Excerpta Medica Foundation produces camera-ready copy for each monthly issue of Epilepsy Abstracts, which includes an index of subjects and authors, and prints and distributes the journal monthly with a cumulative index at the end of the volume. In order to pay for the production of the camera-ready copy, the printing, and distribution, the Excerpta Medica Foundation sells subscriptions to recover the cost of production of camera-ready copy, printing, and distribution.

Course of Contract: Subscriptions to Epilepsy Abstracts, each at an annual cost of $46.00, have been acquired from interested persons by Excerpta Medica at a satisfactory rate. Interest in the publication continues at a high level.

Proposed Course of Contract: Monthly issues have been distributed as scheduled; computer tapes have been delivered in accordance with the contract. This tape will be added to the Epilepsy Abstracts Retrieval System (EARS) data base.

CONTRACT NARRATIVE
Applied Neurologic Research Branch--Section on Epilepsy
July 1, 1974--July 1, 1975

ABBOTT LABORATORIES (N01-NS-3-2314

Title: Synthesis and Testing of New Anticonvulsants

Contractor's Project Director: N. P. Plotnikoff, Ph.D.

Current Annual Level: $102,687

Objectives: To synthesize a new series of benzopyran compounds and evaluate their potential anticonvulsant spectra in animals. The best candidate compounds will be evaluated for pharmacologic properties.

Course of Contract: The contract was initiated on June 15, 1973, and work started immediately therafter. Approximately 120 compounds have been synthesized and evaluated for anticonvuslant activity in mice and rats. Several of these compounds are now undergoing final pharmacologic evaluation.

Proposed Course of Contract: This study will continue with limited synthesis and detailed pharmacologic evaluation of anticonvulsant activity of the most promising compounds.

CONTRACT NARRATIVE
Applied Neurologic Research Branch--Section on Epilepsy
July 1, 1974--June 30, 1975

NATIONAL BUREAU OF STANDARDS (RFP-NINDS-74-02)

Title: Anticonvulsant Reference Standards in Biologic Matrix

Contractor's Project Director: Robert Schaffer, Ph.D.

Current Annual Level: $338,600

Objective: To develop standard reference materials for diphenylhydantoin, primidone, phenobarbital and ethosuximide in a biologic matrix.

Course of Contract: This project was initiated on June, 1974, as an inter-agency agreement. Four anticonvulsant drugs were obtained from commercial sources and evaluated for purity. Various methods of analysis are being used e.g., NMR, mass spectrometry, liquid chromatography, to purify the compounds.

Proposed Course of Contract: This project will continue for 1 more year at which time the standard reference materials will be incorporated into a biologic matrix, e.g., plasma. The concentrations of the four anticonvulsants will be determined accurately. Stability studies will be done, as well as field evaluation of the product, in several analytical laboratories.

CONTRACT NARRATIVE
Applied Neurologic Research Branch--Section on Epilepsy
July 1, 1974--June 30, 1975

Contractor		Project Director	Annual Level
Univ of Minnesota	(NO1-NS-4-2310)	R. Gumnit, M.D.	$74,265
Albert Einstein Col of Med	(NO1-NS-4-2311)	A. Rowan, M.D.	89,658
Vanderbilt Univ	(NO1-NS-4-2312)	W. McLain, M.D.	70,749
Univ Utah	(NO1-NS-4-2313)	D. Woodbury, Ph.D.	87,304
Presbyterian Hosp Dallas	(NO1-NS-4-2314)	R. North, M.D.	51,712
Univ of Washington	(NO1-NS-4-2315)	A. Ward, M.D.	71,162
Good Samaritan Hosp Portland	(NO1-NS-4-2316)	R. Dow, M.D.	86,435
Epilepsy Center of Michigan	(NO1-NS-4-2317)	E. Rodin, M.D.	83,043
Univ Iowa Hosp	(NO1-NS-4-2318)	R. Fincham, M.D.	59,014
Univ Va Med Center	(NO1-NS-4-2319)	F. Dreifuss, M.D.	78,224
Johns Hopkins Univ	(NO1-NS-4-2320)	J. Freeman, M.D.	63,128

Title: Feasibility Study for Comprehensive Epilepsy Program

Objectives: To determine the feasibility of successfully establishing a comprehensive epilepsy program for a defined geographic location.

Course of Contract: Each contractor has made a feasibility determiantion for its location. The means of coordinating existing services was developed; possible clinical epilepsy research was investigated. Inpatient facilities for diagnosis and treatment of difficult-to-control patients was explored.

Major Findings: Determinations of feasibility for conduct of a comprehensive epilepsy program are being made for a definite geographic area by each of the 11 contractors. A request for proposals was issued in February, 1975, for conduct of a comprehensive program. Ten of the 11 contractors responded with a proposal.

Proposed Course: The contracts end in June, 1975, with the completion of the feasibility study. Some of the contractors will require three months' additional time, without additional funds, to complete their analysis.

CONTRACT NARRATIVE
Applied Neurologic Research Branch--Section on Epilepsy
July 1, 1974--June 30, 1975

UNIVERSITY OF CALIFORNIA AT LOS ANGELES (NO1-NS-4-2330)

Title: Metabolic Studies of DMMP

Contractor's Project Director: Donald Jenden, M.D.

Current Annual Level: $88,818

Objectives: To develop methodology for the analysis of dimethoxymethyl phenobarbital (DMMP) and its metabolites and then study the metabolism and pharmacokinetics of DMMP in epileptic patients.

Course of Contract: The contract was initiated in June,1974, and work began thereafter. Gas-chromatography mass-spectrometry has been adapted to the analysis of DMMP and its metabolites to allow for the quantitation and eludication of structure of small amounts of drug. Pilot patient study is now in progress where DMMP is added to the anticonvulsant regimen.

Proposed Course: The study will continue for an additional 6 months at which time the pharmacokinetic data will be gathered.

CONTRACT NARRATIVE

Applied Neurologic Research Branch--Section on Epilepsy
July 1, 1974--June 30, 1975

UNIVERSITY OF UTAH (NO1-NS-5-2302)

Title: Initial Pharmacologic Development of New Drugs

Contractor's Project Director: Ewart A. Swinyard, Ph.D.

Current Annual Level: $129,000

Objective: To determine the anticonvulsant properties of novel organic compounds in mice at various times following intraperitoneal administration.

Course of Contract: Compounds are received by NINCDS from academic and industrial sources, and then sent to the University of Utah. The levels of anticonvulsant and neurotoxicity activities are determined. For those compounds which are found to be active, the ED_{50} and TD_{50} are determined. The pharmacologic data is then provided to NINCDS where it is reviewed and analyzed. The submitters of compounds are then informed of the results for their use for further synthesis. Compounds which are shown to be active will be selected for future development.

Proposed Course of Contract: This program began Jan. 1, 1975, and will continue through June, 1976; approximately 1500 compounds will be screened for anticonvulsant activity during 18 months.

CONTRACT NARRATIVE
Applied Neurologic Research Branch--Section on Head Injury and Stroke
July 1, 1974--October 20, 1974

UNIVERSITY OF NEW MEXICO (NIH-71-2316)--Coordinating Center
VIRGINIA COMMONWEALTH UNIVERSITY (NIH-71-2315)
OHIO STATE UNIVERSITY (NIH-71-2317)
UNIVERSITY OF CALIFORNIA, SAN DIEGO (NIH-71-2318)
NEW YORK UNIVERSITY (NIH-71-2319)
NORTHWESTERN UNIVERSITY (NIH-71-2320)
UNIVERSITY OF UTAH (NIH-71-2322)
COLLEGE OF PHYSICIANS & SURGEONS-COLUMBIA UNIVERSITY (NIH-72-2312)

Title: Collaborative Study of Cerebral Death

Project Coordinator: A. Earl Walker, M.D. (UNM)

Project Directors: Cary Suter, M.D. (VCU)
Norman Allen, M.D. (OSU)
Reginald G. Bickford, M.D. (UCSD)
Julius Korein, M.D. (NYU)
Benjamin Boshes, M.D. (NU)
Donald R. Bennett, M.D. (UU)
Eli Goldensohn, M.D. (CPS)

Current Annual Level: (0)

Objectives: The overall objectives of the collaborative study of cerebral death were 1) to verify or modify the current clinical and electroencephalographic criteria for cerebral death in relation to patient age and cause of coma; 2) to determine the minimal time that clinical and EEG criteria must be operative to indicate cerebral death in relation to age and cause of coma; 3) to assess the neurologic deficit of patients who improve after having fulfilled all criteria for some length of time; and, finally, 4) to characterize abnormal EEG patterns seen prior to electrocerebral silence.

Major Findings: The study is completed, and 503 cases have been analyzed. The report summarizing the results of the study has been received and is in review by NINDS staff.

In the apneic, unresponsive patient the results show that a one-hour flat EEG predicts death to a high degree of accuracy. However, "respirator brain" was not present in all patients meeting the Harvard Criteria, and, therefore, cerebral death remains a clinical syndrome without a standard pathological diagnosis.

There have been two cases of drug intoxication in which the EEG's have been flat and subsequent recovery achieved. In view of this fact, any future studies will have to provide an alternative means of identifying the drug-intoxicated cases. By further requiring fixed and dilated pupils, these two cases would not have been exceptions. However, back-up tests of cerebral blood flow estimations need to be used. A noninvasive test of circulation to the brain, already proven feasible, is under study at New York University and University of Minnesota for reliability and validity, using aortocranial angiography of the brain as the standard control.

The final report of the collaborative project concludes that although death under certain circumstances may be predicted with varying degrees of confidence, definitive criteria for brain death cannot be established on a statistical basis.

Significance to NINCDS Program and Biomedical Research: Considerable controversy still exists over the legal, moral, and scientific definitions of death. The criteria used for the diagnosis of brain death vary in the world literature. The need for reliable uniform criteria still exists in order to spare families the emotional stress and financial burden of unnecessary delays in pronouncement of death, and, at the same time, to protect against undue haste in cessation of efforts of resuscitation. European criteria include assessment of blood flow by aortocranial angiography in primarily brain-damaged patients while the Harvard Criteria which do not require blood flow assessment remain the most widely accepted norms in this country. This study points out the need for establishing morphological criteria for respirator brain and irreversible anoxia as well as the need to quickly identify patients who have taken depressant drugs in the absence of clinical history. While the investigators conclude that some quick noninvasive blood flow measurement should be developed to increase the accuracy of prediction of death, the study also points out the need for standardization and dissemination of drug screening procedures.

Several important manuscripts are in editorial review (selected for presentation at scientific meetings) or in press including:
a) Respirator Brain: Report of a Survey and Review of the Current Concepts, Moseley JI, Molinari GF, Walker AE.
b) Brain Death: Report of the NINDS Collaborative Study. I - Study Design, Demography, and Etiology. Coordinator, Project Directors and Statisticians. Brain Death: Report of the NINDS Collaborative Study. II - Coordinator, Project Directors and Statisticians.
c) Cerebral Death: A Critique of Criteria, Walker AE, Molinari GF.

Additional publications have appeared or are in press from participants in this study: Pulsatile Echoencephalography as an Indicator of Cerebral Circulation, An Atlas of EEG Changes in Brain Death Suspects, The Clinical Findings in Brain Death Suspects, and The Neurology of the Dying Process.

Publications: Walker, A.E., Diamond, E., and Moseley, J.I.: Neuropathological findings in irreversible coma. J Neuropathol Exp Neurol. (in press)

CONTRACT NARRATIVE
Applied Neurologic Research Branch--Section on Head Injury and Stroke
July 1, 1974--June 30, 1975

INDIANA UNIVERSITY FOUNDATION (NIH-72-2324)

Title: A Study of the Hospital Frequency and Character of Transient Ischemic Attacks--Mark L. Dyken, M.D., Principal Investigator

Current Level: $247,406 (21 months)

Collaborating Centers:
University of Maryland
University of Mississippi
Institute of Medical Sciences
University of Washington
Massachusetts General Hospital
Indiana University

Project Directors:
Thomas R. Price, M.D. (Md)
Armin F. Haerer, M.D. (UMiss)
Philip R. Calanchini, M.D. (IMS)
Phillip D. Swanson, M.D. (UW)
David C. Poskanzer, M.D. (MGH)
Mark L. Dyken, M.D. (Ind)

Objectives: The objectives of this study are to determine and describe prospectively the relative frequency of each of the etiologies of transient ischemic attacks (TIA's), to classify TIA syndromes, and to establish the frequency and accuracy of diagnosis for each specific TIA syndrome as determined through short term follow-up. The study is limited to patients presenting for hospitalization or hospital outpatient clinics with a presumptive diagnosis of transient ischemic attack.

Major Findings: This study has achieved all of its initial goals and objectives in that it has established the frequency with which TIA like symptoms present in both ambulatory and in-patient services at demographically selected regional medical centers. In excess of 1300 patients have been accessioned and are in the short term (one-year) follow-up period. Preliminary data analyses indicate that a) the initial clinical impression of TIA is erroneous approximately 25 percent of the time; b) a broad spectrum of unrelated but specific diseases present with transient focal neurological symptoms, including partial epileptic seizures, subdural hematomas, brain tumors and primary cardiac dysfunctions; c) the vast majority of confirmed TIA symptoms persist for less than 8 hours (85 percent), while the mode for duration of attacks is less than 2 hours (45 percent approximately); d) initial clinical impressions of TIA in the carotid arterial system are more accurate and reliable than initial impressions of vertebrobasilar ischemic attacks.

Significance to NINCDS Program and Biomedical Research: A data base for evaluating the character, frequency and prognostic significance of transient ischemic attacks will be provided by this project. The findings will influence the interpretation of data from past and future studies of the epidemiology and therapy of TIA. A functional or descriptive definition of TIA's may be developed from this data base which will be of great importance to future studies requiring selection criteria of high risk candidates for microvascular anastomic surgery. Most individual ischemic episodes persist for time periods less than the usual latency from ictus through transportation to hospitals and admission procedures. This probably accounts for the progression in the degree of sophistication and reliability of recorded observations of TIA phenomena. Most events are witnessed only by lay observers outside of hospitals; those lasting somewhat longer may be witnessed by emergency room triage officers and housestaff; while only the longest events are ever seen by the senior clinical investigators.

This study will provide guidelines for the classification of histories of TIA symptoms for reliability and validity. Such a system is critical for the interpretation of results of intervention, medical or surgical, in the natural history of TIA-like symptoms.

Proposed Course of Contract: The contract has been reviewed by the Advisory Group to the Section on Head Injury and Stroke and was thought to have achieved all of its goals and objectives. The project has been awarded an extension in time and phase-out funds to permit completion of data analyses, scientific publication and final reporting to the NINCDS by December 30, 1975.

CONTRACT NARRATIVE

Applied Neurologic Research Branch--Section on Head Injury and Stroke

July 1, 1974--June 30, 1975

UNIVERSITY OF WASHINGTON (NIH-72-2326)

Title: Human Responses to Head Injury

Contractor's Project Director: Ralph M. Reitan, Ph.D.

Current Level: $271,274 (36 months)

Objectives: 1. To describe prospectively the recovery pattern of psychological function after brain trauma. 2. To establish the time course over which recovery occurs. 3. To identify the sub-tests in a comprehensive neuropsychological battery which have the greatest prognostic value.

Major Findings: Based on work done to date: 1. There has been a 30 percent loss to follow-up among survivors of acute head injuries. 2. Patients still in follow-up show progress changes toward improvement and unstable EEG patterns at one year. 3. The loss to follow-up rate and small numbers of patients inhibit the prospective study, but the 40 patients still in follow-up constitute a minimal yet statistically valid sample. 4. Neuro-otological workup of patients has not proved valuable in predicting deafness or permanent vestibular dysfunction in head-injured patients. 5. Neurological and EEG examinations are not equally sensitive to formal psychometrics in assessment of recovery and impairments after head injury and consequently lose their value as controls in the chronic stages of recovery.

Significance to NINCDS Program and Biomedical Research: This study will yield late recovery patterns which reputedly may require up to two years of convalescence for completion. The study is complementary to numerous other program projects addressing prognosis for survival after coma and the quality of functional recovery after head injury. Of particular note is a disturbing tendency for certain patients in the prospective sample to lose psychological functions gained during early convalescence during the second year of follow-up.

Proposed Course: Due to small numbers, statistical significance of trends noted in the prospective subjects will be tested against Dr. Reitan's voluminous data bank of retrospective material. In addition, as a cross validation procedure on all parameters tested in the prospective sample, objective measurement of pathology would be highly desirable. Such measurement is now possible in living patients through application of computer assisted x-ray tomographic techniques (CAT scans). A one-year phase-out period is planned in order to permit two years of follow-up for every patient, including the last entered, and CAT scans will be obtained on every surviving patient immediately after the last psychometric evaluation. Successful completion of this contract is expected after one additional year's effort.

CONTRACT NARRATIVE
Applied Neurologic Research Branch--Section on Head Injury and Stroke
July 1, 1974--June 30, 1975

HOWARD UNIVERSITY COLLEGE OF MEDICINE (NO1-NS-4-2329)

Title: A Study of Prognosis after Stroke

Project Director: Don H. Wood, M.D.

Total Amount Funded: $140,607

Objectives: 1. To provide continuous neurological observations over a six-month period after stroke, beginning with admissions to acute treatment facility and concluding with functional assessment of quality of recovery. 2. To test prospectively in a longitudinal study the accuracy and reliability of clinical signs and symptons reputed to have prognostic value from retrospective surveys.

Major Findings: As of February 28, 1975, 51 patients had been screened of whom 26 met entrance criteria to the longitudinal study protocol. The most prevalent reasons for failure to meet entrance criteria were a) inordinate latency from ictus to hospital admission, b) chronic strokes readmitted for intercurrent respiratory, urinary infections, etc., but presenting as recrudescence of stroke like symptoms, and c) transient ischemia attacks and/or minor strokes clearing without deficits.

Significance to NINCDS Program and Biomedical Research: This study is a necessary step toward achievement of the goals and objectives of the Head Injury and Stroke Section's program, aimed at improvement in the quality of life of survivors. From retrospective data analyses, coma or the state of consciousness at the outset of strokes is the most reliable prognostic indicator of both survival and the quality of recovery. This study interrelates closely with the concurrent study "Prediction of Outcome of Patients in Coma" (vi) in that it takes into account the multivariants affecting outcome, while the Cornell University project attempts to precisely define and quantitate degrees of coma as well as its duration.

Proposed Course: The project, negotiated and approved for two years, will be completed within the calendar year 1976. A no-cost extension is anticipated due to lower than anticipated patient accession rate and the logistical delays encountered in transferring of patients and resources to the new Freedman's Hospital in March 1975.

CONTRACT NARRATIVE
Applied Neurologic Research Branch--Section on Head Injury and Stroke
July 1, 1974--June 30, 1975

CORNELL UNIVERSITY MEDICAL COLLEGE (N01-NS-4-2328)

Title: Prediction of Outcome of Patients with Coma

Project Director: Fred Plum, M.D.

Current Annual Level: $195,179

Objectives: 1. To validate a pretested semiquantitative system for grading the severity of coma. 2. To test the predictive power of graded levels of coma on short term prognosis (six months).

Major Findings: Case accession rate has been satisfactory to date at the two centers studying medical (nontraumatic) coma, namely, Cornell University Medical Center and Royal Victoria Infirmary, Newcastle, England. In 115 patients followed for a minimum of three months, the oculovestibular reflex gave the single best accuracy of prediction.

Accession rate has been slow at two centers selected for the study of traumatic coma at University of Southern California, but at Glasgow 150 neurosurgical patients have been studied.

Significance to NINCDS Program and Biomedical Research: Successful completion of this study will produce a validated, field tested, semiquantitative system for grading the severity of coma. The contributory components to the grading system are routine bedside assessments which do not require sophisticated equipment or technical skill to administer. This system will find broad utility and permit standardization of the clinical monitoring of patients presenting at stroke and head injury care facilities. Moreover, the potential for prediction of quality of outcome is of enormous significance and may have broad though controversial social impact. This study augments the fund of knowledge accumulated in the NINCDS Collaborative Study of Brain Death in that the patients under study are not apneic and quality of survival is precisely defined and categorized into four levels.

Proposed Course: It is anticipated that this project will be supported to completion (target date June 30, 1976).

CONTRACT NARRATIVE
Applied Neurologic Research Branch--Section on Head Injury and Stroke
July 1, 1974--June 30, 1975

UNIVERSITY OF MINNESOTA (NO1-NS-4-2306)

Title: Comparison of Isotope - Bolus Technique with Contrast and Isotope Angiography

Project Director: Shelley N. Chou, M.D.

Current Annual Level: $69,500

Objectives: To test the validity and reliability of a simple noninvasive method of assessment of cerebral circulation as a measure of cerebral perfusion compared to aortocranial positive contrast angiography and isotope angiography using a gamma camera.

Major Findings: Accession rate of head-injured patients and brain death suspects has fallen precipitously at Minnesota as a result of reduced speed limits imposed after energy crisis of 1972-73. To date patients studied show isotope angiography (Y-camera) to be as reliable as contrast angiography. Special portable equipment, using three probes as specified in proposal was installed and operational as of November 1974. Initial studies show equal validity and reliability among all three methods.

Significance to NINCDS Program and Biomedical Research: Quantitative blood flow methods have been in use for more than 25 years, but none have found clinical usefulness and general acceptance as reliable parameters of pathophysiology in humans. The cerebral angiogram remains the norm for diagnostic cerebral circulation studies in man. Although not quantitative, 4-vessel aortocranial angiography has become a useful index of cerebral viability in Europe. Because of the additional risk of transporting moribund patients to the x-ray suite and the hazard inherent in invasive angiography itself, the criterion of cerebral perfusion has not been exploited in this country as the fail-safe method of determining death.

This study proposes to demonstrate concordance between perfusion of the cerebral vascular tree by an externally detected passage of a nondiffusible isotope, ^{99m}Tc pertechnetate, between two laterally placed sodium iodide crystals and positive contrast angiography. If equivalency between the methods can be established, the isotope technique which requires only an intravenous injection will have wide clinical application in brain death, since only portable equipment is required.

Proposed Course: Because of the delay in delivery of necessary instrumentation and lower than anticipated accession rate, this contract will require extension in time without additional funds.

CONTRACT NARRATIVE
Applied Neurologic Research Branch--Section on Head Injury and Stroke
July 1, 1974--June 30, 1975

NEW YORK UNIVERSITY (N01-NS-4-2307)

Title: Study of Intravenous Isotope Scintillation Angiography of the Brain in Man

Project Director: Philip Braunstein, M.D.

Current Annual Level: $111,500

Objectives: To validate a radionuclide scintillation detector technique as a qualitative test of cerebral circulation.

Major Findings: To date 12 patients with electrocortical silence by EEG have been studied with both the portable scintillation detection equipment and 4-vessel aortocranial angiography using a power injector. To date there are no discrepencies among nonfilling by contrast angiography, flat isotope "bolus curves" and ECS. "Intermediate curves" may be associated with nonfilling by angiography, however. These findings indicate that errors inherent in the technique favor the conservative interpretation of cerebral viability but that the test is a valid indicator of no cerebral perfusion.

Significance to NINCDS Program and Biomedical Research: Quantitative blood flow methods have been in use for more than 25 years, but none have found clinical usefulness and general acceptance as reliable parameters of pathophysiology in humans. The cerebral angiogram remains the norm for diagnostic cerebral circulation studies in man. Although not quantitative, 4-vessel aortocranial angiography has become a useful index of cerebral viability in Europe. Because of the additional risk of transporting moribund patients to the x-ray suite and the hazard inherent in invasive angiography itself, the criterion of cerebral perfusion has not been exploited in this country as the fail-safe method of determining death.

This study proposes to demonstrate concordance between perfusion of the cerebral vascular tree by an externally detected passage of a nondiffusible isotope, ^{99m}Tc pertechnetate, between two laterally placed sodium iodide crystals and positive contrast angiography. If equivalency between the methods can be established, the isotope technique which requires only an intravenous injection will have wide clinical application in brain death, since only portable equipment is required.

The reliability and potential sources of error in the isotope bolus detection system will be completely delineated, analyzed and reported to the Institute upon completion of this study. It is important to note that this project is not a study of brain death per se, but successful completion of this test will provide validity and reliability of a very cogent tool to be used in pronouncing death based upon central nervous system criteria.

Proposed Course: Because of the delay in delivery of necessary instrumentation and lower than anticipated accession rate, this contract will require extension in time without additional funds.

MAYO MEDICAL SCHOOL (N01-NS-4-2333)

Title: Study of Transverse Axial Tomography in Cerebrovascular Disease

Project Director: Otis W. Houser, M.D.

Current Annual Level: $125,540

Objectives: 1) To test the spatial resolution power of computer assisted x-ray tomography. 2) To test the latency for alteration of radio absorbency of ischemic cerebral tissue.

Major Findings: Research program is progressing satisfactorily. The accession rate of study patients varies between 15 and 20 new cases per month, and at present, approximately 100 patients have been studied with at least two scans. An initial study is obtained within 24 hours of admission followed by a repeat examination seven days later.

Significance to NINCDS Program and Biomedical Research: Successful completion of this study will provide the NINCDS with a true test in terms of real pathology of the spatial resolution, temporal latency, and thereby the limits of applicability of this new technology to clinical stroke problems. While a body of literature has developed very quickly on the sensitivity of CAT scans to intracerebral hemorrhages, little has been spontaneously reported on the utility of the equipment in detecting and localizing acute ischemic processes. Data and conclusions from this study will provide guidelines for the appropriate application of CAT techniques in existing and future comprehensive cerebrovascular disease research programs.

Proposed Course: This two-year project will be completed by the end of FY 1976.

CONTRACT NARRATIVE
Applied Neurologic Research Branch--Section on Head Injury and Stroke
July 1, 1974--June 30, 1975

DUKE UNIVERSITY MEDICAL CENTER (NO1-NS-4-2337)

Title: Detection of TIA in an Elderly Community

Project Director: Albert Heyman, M.D.

Current Annual Level: $90,491

Objectives: 1. To develop, field test and validate a self-administered questionnaire for the identification of previously undiagnosed transient ischemic attacks (TIA) in the community. 2. To assess the incidence and prevalence of TIA in the high stroke risk populations of aging Americans in a demographically selected cross section of the country.

Major Findings: The questionnaire was pretested, revised and administration procedures standardized in subsamples in Durham, North Carolina and New Haven, Connecticut.

The accuracy and validity of the instrument have been established as acceptable for a screening tool by follow-up contact interviews by qualified neurologists.

Surveys have been completed in two major U.S. cities, Boston, Massachusetts, and New Haven, Connecticut.

Significance to NINCDS Program and Biomedical Research: This study complements and extends the effort initiated in the collaborative hospital-based study of TIA aimed at early detection of the stroke prone. The hospital study questions the accuracy of the initial diagnosis, grades the quality and reliability of individual historical symptoms and symptom complexes in terms of short-term prognosis and final diagnosis in a preselected population presenting at or referred to university hospitals. The questionnaire developed surveys in a high risk population, unselected except by age, the most prevalent symptoms suggesting TIA and those commonly confused with TIA. Special inquiry is being made into the incidence, prevalence and significance of a reputedly common and troublesome symptom among aging persons, namely, non-vertiginous dizziness.

Proposed Course: This project to be completed during FY 1976.

Project No. Z01 NS 01933-05 ANR
1. Applied Neurologic Research Branch
2. Epilepsy Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974--June 30, 1975

Project Title: Quantitation of clinical manifestations of spike-wave activity by a reaction time method
Previous Serial Number: NDS (CF)-71 AN 1933
Principal Investigator: J. Kiffin Penry, M.D.

Other Investigators: Susumu Sato, M.D.
Fritz E. Dreifuss, M.D.

Cooperating Unit: Department of Neurology
University of Virginia School of Medicine
Charlottesville, Virginia

Man Years:

Total:	0.5
Professional:	0.25
Other:	0.25

Facilities and Equipment:

Electroencephalographic equipment and space for testing were provided by the University of Virginia Hospital at Charlottesville. The reaction time equipment including seizure detection device and digital timer were designed and built by the Section on Epilepsy. The video-recording apparatus was provided by the Section on Epilepsy.

Project Description:

Objectives: The purpose of this study is to determine whether reaction time in absence patients is or is not impaired in a gradual fashion from the point of spike-wave initiation as has been suggested by some authors but disputed by others. There is some evidence for a "trough-like" pattern decrease of consciousness. The onset of decrease clinical functions during spike-wave paroxysms is evaluated by the reaction time method.

Methods Employed: A device is employed which gives instantaneous recognition by voltage criteria that a spike-wave burst has started. This burst is of much higher than normal background, and this factor alone is used to electronically trigger the reaction timer. On instantaneous recognition the reaction timer is triggered and a tone is delivered to the subject. The subject responds by turning off the high pitch tone with a telegraph key. Between paroxysms the patient is maintained in a state of alertness by a program of approximately 10 random stimuli per minute. All the data is collected by television, including a portion of the screen reserved for the reaction time from the digital clock. There is no age limit in selecting patients, but they must all have spike-wave paroxysmal discharge. A second group of patients was studied with the apparatus altered slightly so that the auditory stimulus was delivered 0.5 seconds into the seizure in order to see if responsiveness becomes less as the seizure progresses. Oscillographic displays from magnetic tape recordings of spike-wave paroxysms revealed shifting asymmetries.

Major Findings: The findings of the first group of patients suggest that some ability to respond early during the paroxysmal burst is maintained; this responsiveness is frequently not seen 1-2 seconds after onset. Analysis of responsiveness during short bursts suggests that patients may retain a normal reaction time during such paroxysms.

Significance to Biomedical Research and to the Program of the Institute: This study has applied video recording techniques and sophisticated electronic methods to improve the quality of clinical research. Specifically, this study is an analysis of the relation of the patient's behavior to his EEG during paroxysmal electroencephalographic events. An understanding of this relationship is important--not only as a guidepost for further research in the mechanism of epilepsy, but also in determining the day-to-day therapeutics of the epileptic patient.

Proposed Course: The study will be continued with additional patients in the coming fiscal year. The degree of generalization of the spike-wave paroxysms will be measured by computer. The computer-aided analysis has been delayed while a new minicomputer has been acquired and placed in working order.

Keyword Descriptors: Absence seizures, electroencephalography, spike-wave, reaction time.

Project No. Z01 NS 02097-04 ANR
1. Applied Neurologic Research Branch
2. Epilepsy Section
3. Bethesda, Maryland

PHS--NIH
Individual Project Report
July 1, 1974--June 30, 1975

Project Title: Diagnostic value of prolonged telemetered EEG in epilepsy
Previous Serial Number: NDS (CF)-72 AN 2097
Principal Investigator: J. Kiffin Penry, M.D.

Other Investigators: William L. Brannon, Jr., M.D.

Cooperating Unit: Department of Neurology
Naval Medical Center
Bethesda, Maryland

Man Years:

Total:	0.2
Professional:	0.1
Other:	0.1

Facilities and Equipment:

The telemetry equipment and FM tape recorder were provided by the Section on Epilepsy. Research was conducted at the Department of Neurology, Naval Medical Center, Bethesda, Maryland.

Project Description:

Objectives and Methods: To develop a means of detecting and recording interictal paroxysmal abnormalities (epileptiform) in the EEG of patients who have suffered a clinical convulsion. Routinely, a 20-30 minute electroencephalogram, which includes hyperventilation and photic stimulation, will detect interictal EEG abnormalities in 30-50% of the patients who suffer convulsions; there remains the other 50% of the patients who are at risk for recurrent convulsions and may be suffering the onset of epilepsy. Traditionally these patients must await recurrence of seizures to make a diagnosis, or repeating EEG determinations in the hope of catching an interictal abnormality. A recent study by Johnson, et al. has shown that 40% of the patients who suffer a single generalized tonic-clonic seizure have a recurrence within one year. If these patients could be detected they could be treated and seizure occurrence prevented, without treating those patients who will not have a recurrence.

Therefore, a study has been designed to record a telemetered EEG for 6 hours in order to sample over a period of time longer than the usual 20-30 minutes and during normal activity; in some patients, the latter may evoke interictal paroxysmal abnormalities. The study compares the incidence of diagnostic paroxysmal abnormalities in the 6-hour telemetered EEG with those from routine conventional EEGs.

Course of the Study: To date 30 patients have been studied. Each patient has had a bona fide seizure within 30 days of the recording, and has also had a normal electroencephalogram by ordinary diagnostic workup.

Statistically, it is estimated that from 60-80 patients will be required to prove the hypothesis that the prolonged telemetered EEG during normal behavior will significantly increase the diagnostic ability over that of a single EEG after the first seizure. The flow of patients has been somewhat less than desired.

Major Findings: In the first 30 patients accessioned, three have had diagnostic abnormalities on the 6-hour telemetered EEG which were not recorded in the routine EEG. The accessioning of patients will continue over another 10 months.

Significance: The ability to detect and record diagnostic epilepsy format abnormalities in the EEG after a single seizure will aid in the early treatment and long-term prognosis of patients who suffer their initial seizure.

Keyword Descriptors: Electroencephalography, telemetry, seizures, interictus.

Project No. Z01 NS 02098-02 ANR
1. Applied Neurologic Research Branch
2. Section on Epilepsy
3. Bethesda, Maryland

PHS--NIH
Individual Project Report
July 1, 1974--June 30, 1975

Project Title: The Monaural Auditory Evoked Potential as a Measure of Anticonvulsant Drug Effect on Brain Function.

Previous Serial Number: NDS (CF)-74 AN 2098

Principal Investigator: Jonathan R. Wolpaw, M.D.

Other Investigator: J. Kiffin Penry, M.D.

Man Years:

Total:	0.5
Professional:	0.3
Other:	0.2

Facilities & Equipment: Laboratories of NINCDS, NIH

Project Description:

Objectives: To develop the ipsilateral-contralateral peak latency difference previously noted by others in the monaural auditory evoked response as a measure of acute and chronic anticonvulsant effect on central nervous system function.

Major Findings: Through an extensive study in normal control, we further define the origin and magnitude of the previously reported ipsilateral-contralateral peak latency difference. In pilot studies, moderate doses of ethanol and caffeine increased the ipsilateral-contralateral peak latency difference, often giving differences significantly higher than control values. At present, data is being collected from patients before and after initiation of anticonvulsant therapy. Results from a small number of patients allow no definite conclusions at this point, but it appears that phenobarbital gives abnormally high ipsilateral-contralateral peak latency differences.

Proposed Course: Continued collection of data from patients pre- and post-initiation of anticonvulsant therapy, continued follow-up of patients already in study if possible, and continued ancillary control studies to further define the nature of the measurement and to arrive at the best possible method of measuring it.

Keyword Descriptors: Auditory evoked potentials, brain function.

Project No. Z01 NS 02186-01 ANR
1. Applied Neurologic Research Branch
2. Section on Epilepsy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974--June 30, 1975

Project Title: A Pilot Study of Complex Partial Seizures
Previous Serial Number: None
Principal Investigator: Roger J. Porter, M.D.

Other Investigators: J. Kiffin Penry, M.D.
John M. Van Buren, M.D.

Cooperating Units: Surgical Neurology Branch, NINCDS
Television Engineering Section, Clinical Center, NIH

Man Years:
Total: 0.90
Professional: 0.50
Other: 0.40

Facilities and Equipment: The EEG telemetry equipment, including transmitter, receiver, oscilloscope monitor, and FM tape recorder provided by the Section on Epilepsy. The video equipment including cameras, special effects generator, video timer, monitors, and recorders provided by the TV Engineering Section. A part-time technician provided by the Section on Epilepsy for the telemetry system and for video recording. The TV Engineering Section gave technical support for video recorders located in the basement.

Project Description: This study utilizes methods which are documented for evaluating absence seizures in a pilot study of complex partial seizures to: (1) Establish the ability of the present techniques of video recording and prolonged telemetered recording to determine a patient's seizure frequency; (2) Establish the ability of these techniques to classify seizure disorders other than absence; (3) Establish guidelines for patients who may be considered candidates for surgical management of their seizure disorder.

Methods Employed: Patients from the Surgical Neurology Branch are studied during three consecutive days. Intensive observation by a trained person, telemetered EEG, and 6 hours of video recording are done each day. During recording hours, the patient is continuously available for monitoring on the ward. Pre and post-operative recordings of the patients are made. The telemetered EEG tapes are returned to the laboratory of the Section on Epilepsy where hard-copy tracings are made, and the video recordings are edited and the tapes reused.

Major Findings: Although the pilot study has not been completed, some observations are apparent: (1) The present video techniques are valuable in determining the qualitative nature of a patient's seizures, and are of some benefit clinically, especially for localization and determination of hysterical attacks; (2) Further investigation on differences in classification of seizure disorders is proceeding. An international workshop will be held so that others with recorded data on complex partial seizures may share data and impressions. Recorded seizures from this pilot study will be an integral part of this workshop; (3) It has been shown that surgical management of patients with epilepsy can be altered by the above techniques, although clear-cut guidelines have not yet emerged.

Significance to NINCDS Program and Biomedical Research: This pilot study has documented the usefulness of techniques of video and telemetered EEG recordings in the evaluation of complex partial seizures. It has provided a framework for a more extensive study of these techniques which will begin June,1975.

Keyword Descriptors: Seizures, EEG telemetry, video recordings.

Honors and Awards: None

Publications: None

Project No. Z01 NS 02187-01 ANR
1. Applied Neurologic Research Branch
2. Section on Epilepsy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974--June 30, 1975

Project Title: Disposition of Kö 1173--a new antiepileptic agent, in mice.
Previous Serial Number: None
Principal Investigator: Roger J. Porter, M.D.

Other Investigator: Harvey J. Kupferberg, Ph.D.

Man Years:
Total: 0.2
Professional: 0.2

Facilities and Equipment: Laboratories of NINCDS

Project Description: To determine the blood and brain concentrations of racemic Kö 1173 and its antipods in mice in relation to its anticonvulsant activity.

Method Employed: Mice were given Kö 1173 [1-(2',6'dimethylphenoxy)-2-aminopropane] intraperitoneally and anticonvulsant activity determined by the maximal electroshock seizure test. Blood and brain levels of Kö 1173 were determined by gas-liquid chromatography. Optically active d-benzyl tartrate salts of Kö 1173 were made as a means of separating racemic Kö 1173 into its optically activity antipods.

Major Findings: The anticonvulsant activity of Kö 1173 in mice is of short duration, and its rate of disappearance from plasma parallels the anticonvulsant activity. Brain levels of Kö 1173 are much higher than plasma levels, due to the high lipid solubility of Kö 1173. The ED_{50} for Kö 1173 at 30 minutes following administration is 10 mg/kg and rises to 43 mg/kg at 3 hours.

Keyword Descriptors: Experimental seizures, mice, Kö 1173, drug metabolism.

Project No. Z01 NS 02188-01 ANR
1. Applied Neurologic Research Branch
2. Section on Epilepsy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974--June 30, 1975

Project Title: Metabolism of Ethotoin in Epileptic Patients

Previous Serial Number: None

Principal Investigator: Harvey J. Kupferberg, Ph.D.

Other Investigators: Wayne D. Yonekawa, M.S.
Fredric K. Cantor, M.D.

Cooperating Units: Georgetown Medical School, Department of Neurology
Veterans Administration Hospital, Washington, D.C.

Man Years:

Total:	0.25
Professional:	0.20
Other:	0.05

Project Description: To determine the metabolic pathways of ethotoin in epileptic patients.

Major Findings: Several metabolites of ethotoin were isolated and identified in urine of epileptic patients receiving 2.0 gms of ethotoin. They are: 5 phenylhydantoin, 3 ethyl -5 parahydroxyphenylhydantoin and its glucuronide, 3 ethyl-5 metahydroxyphenylhydantoin, and 2 phenyl hydantoic acid. The later compound is found only in the R(-) form. The parahydroxyphenyl metabolite of ethotoin is in much greater quantity than the hydroxyphenyl-metabolite. No further metabolites of 5 phenylhydantoin were found.

Proposed Course: This project has been completed.

Keyword Descriptors: Epilepsy, ethotoin, drug metabolism, metabolites.

Project No. Z01 NS 02051 - 03 ANR
1. Applied Neurologic Research Branch
2. Section on Head Injury and Stroke
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Experimental Models of Stroke: Modification of Lesion Size and Character by Combined Pharmacologic and Surgical Intervention

Previous Serial Number: NDS (CF)-73 AN 2051

Principal Investigators: G. F. Molinari, M.D.
J. Fein, M.D. (Albert Einstein University)

Man Years:

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description:

Objective: 1. To establish the maximum duration of cerebral tolerance of ischemic anoxia and to evaluate the association of ischemia and hemorrhage in primate models. 2. To determine the maximum time allowable to begin therapeutic intervention by a number of methods.

Methods Employed: Having established the pathologic anatomy of models of ischemia, acute experiments using hydrogen washout blood flow methods, EEG, oxygen polarography, the early stages of evolution of ischemia have been studied. These parameters will be monitored simultaneously in both primary and secondary zones of disordered circulation before, during and after a) embolectomy and b) middle cerebral-temporal artery anastomoses in dogs.

Major Findings: Models have been developed for segmental cerebral artery occlusion in dogs and primates. Hemorrhagic or bland infarction can be selectively produced depending upon the duration of ischemia prior to reflow through vessels in the ischemic field. Using hemorrhagic infarction as the marker of excessive time lapse, the salutory and pejorative effects of microvascular surgery will be tested under a) N_2O and b) pentobarbital anesthesia.

Significance to Biomedical Research and the Program of the Institute: These experiments are designed to provide basic pathophysiological data related to secondary prevention of stroke. While certain areas of direct traumatic necrosis or total cessation of blood are inevitable, functional sequelae may be minimized by prevention of detrimental secondary vascular events in fields of collateral circulation. Preservation of the collateral field is then the aim of surgical intervention in acute stroke.

These experiments are expected to complement clinical studies of secondary prevention in patients supported by the Section's field research program. Data derived from this study is a vital pretest of efficacy-safety of procedures advocated for use in human stroke victims.

Proposed Course: Under previous interagency agreement (NIH-AFRRI), arrangements with Dr. Fein have produced studies not within the capabilities of this Section's in-house laboratory. A contract with Dr. Fein's institution will be negotiated to continue the ongoing research projects. These include use of dogs for superficial temporal middle cerebral anastomotic surgery studies of oxygen availability and cerebral metabolism following middle cerebral artery occlusion in primates.

Keyword Descriptors: stroke models, barbiturate protection, efficacy of interventive surgery, temporal-middle cerebral anastomosis, cerebral embolectomy

Publications: Fein, J., Molinari, G.F.: Experimental augmentation of regional cerebral blood flow by microvascular anastomosis. J Neurosurg 41:421-426, 1974

Fein, J., Molinari, GF., Parkhurst, L.J.: Augmentation of regional cerebral blood flow by microvascular anastomosis. AFRRI Scientific Report (SR74-12 June, 1974

Project No. Z01 NS 02118-02 ANR
1. Applied Neurologic Research Branch
2. Section on Head Injury and Stroke
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Stroke Models in the Primate--Intracerebral Hematoma

Previous Serial Number: NDS (CF)-74 AN 2118

Principal Investigator: John P. Laurent, M.D.

Other Investigators: G. F. Molinari, M.D.
John C. Oakley, M.D.

Man Years:

Total:	1.5
Professional:	1.0
Other:	0.5

Project Description:

Objective: To develop a method for selectively producing intracerebral hematomas in basal ganglia and deep structures, sparing cortex.

Methods Employed: As developed originally in dogs, a technique utilizing Microfil (R) silicone latex cylinders injected into the internal carotid artery was adapted to the monkey. Randomly certain animals showed intracerebral hematomas contiguous with zones of deep infarction. This was thought to be a preterminal emiphenomenon related to the hypertension reputedly caused by ketamine.

Major Findings: Further testing of the hypothesis of globus, that hematoma, hemorrhagic infarction and ischemia represent a continuum of pathophysiologic events following ischemia, animals were stressed in acute and chronic phases after embolism with a) ketamine, b) hypercapnia, c) norepinephrine and d) angiotensin. Chronic animals stressed with hypercapnia regularly developed intracranial hematomas distal to the site of vascular occlusion. Other stress agents tested produce more extensive infarction in acute animals compared to nonstressed controls and hemorrhagic infarctions in chronic animals. These findings indicate that: 1. Hemorrhage developed during the vasoproliferative phase of ischemia injury (5-6 days); 2. During vasodilation (hypercapnia) and 3. Progressed from the collaterally supplied peri-infarction area into the necrotic core.

Proposed Course: Preliminary pathological results suggest that with manipulation of mean arterial pressure, pCO_2, etc., a basic method has been developed for the first experimental model of intracerebral hematoma and has established a relationship between ischemia and cerebral hemorrhage. This work has been selected for presentation at the AANP Scientific Sessions, June 1975.

Keyword Descriptors: experimental hematoma, hemorrhagic infarction,
vasoproliferation, collateral circulation,
intracranial hypertension

Publication:

Molinari, G.F., Moseley, J.I., Laurent, J.P.: Segmental middle cerebral artery occlusion in primates: an experimental method requiring minimal surgery and anesthesia. Stroke 5-334-339, 1974.

Project No. Z01 NS 02119-02 ANR
1. Applied Neurologic Research Branch
2. Section on Head Injury and Stroke
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Natural History of Experimental Cerebral Infarction in Conscious Monkeys

Previous Number: NDS (CF)-74 AN 2119

Principal Investigator: J. P. Laurent, M.D.

Other Investigators: J. I. Moseley, M.D.
G. F. Molinari, M.D.

Man Years:

Total:	1.5
Professional:	1.0
Other:	0.5

Project Description:

Objective: To establish clinical sequence and tempo of focal cerebral ischemic infarction in the conscious monkey and to correlate site and size of vascular occlusions with distribution and size of brain parenchymal lesions.

Methods Employed: A comprehensive sensory-motor-behavioral neurological examination was standardized for use in primates. Using the cerebral infarction model of preformed silicone emboli placed via internal carotid catherization into middle cerebral artery, immediate repetitive neurological exams were conducted. Anatomical and pathological specimens were harvested for correlation with the neurological status. All experiments were conducted in animal titrated to the level of "dissociate anesthesia" using ketamine. This permits humane analgesic treatment of animals while observing onset and progression of the acute natural history of strokes.

Major Findings: Proximal or distal middle cerebral artery occlusion resulted in distinctive clinicopathological patterns.

Significance to Biomedical Research and the Program of the Institute: These experiments are designed to provide basic anatomical and pathophysiological data related to acute stroke.

Proposed Course: Project concluded - January 1975. Manuscript submitted to Journal of Neurosurgery still in review. "Clinicopathological Validation of a Stroke Model in the Conscious Primate" Laurent, Molinari, Moseley.

Also a teaching demonstration film entitled "Pathogenesis of Cerebral Infarction in the Conscious Monkey (An Experimental Technique)" (Laurent, Moseley, Molinari) has been used in several lecture presentations outside of NIH.

Keyword Descriptors: experimental models, stroke model, clinical pathogenesis, ketamine

Project No. Z01 NS 02120-02 ANR
1. Applied Neurologic Research Branch
2. Section on Head Injury and Stroke
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Evaluation of the Role of Barbiturate in Treating Acute Cerebral Infarction

Previous Serial Number: NDS (CF)-74 AN 2120

Principal Investigator: G. F. Molinari, M.D.

Other Investigators: John P. Laurent, M.D.
John C. Oakley, M.D.

Man Years:

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description:

Objectives: 1. To determine in the primate whether a short acting barbiturate given for 12 hours beginning within 30 minutes after embolic occlusion of the middle cerebral artery reduces the clinical and/or pathological sequelae observed in the first five days after the infarct. 2. To establish the pathophysiologic basis for the observed protective effect by continuous monitoring of superficial and deep cerebral blood flow and EEG.

Rationale: Cerebral infarction might be delayed by reducing metabolic demand while secondary supply sources improve or are restored. Hydrogen polarographic blood flow methods permit separation of blood flow values into cortical and basal ganglia components.

Major Findings: Compared to a control group, animals treated with barbiturate regained grasp, movement, and some strength in affected limbs. Untreated animals did not improve measurably in these areas during the five-day observation period. Pathologically, the untreated monkeys have both cortical and deep infarction, while treated animals have relative cortical sparing. Furthermore, the morphological changes due to ischemia develop at a slower rate in treated animals. Physiologically, CBF changes occur over the same time axis after embolism in barbiturate and ketamine treated groups. EEG changes, however, are more prominent in ketamine animals due to the higher amplitudes and baseline frequencies in pre-embolic control.

Significance to Biomedical Research and the Program of the Institute: This work partly confirms previous work done in the dog and rabbit. Although more evaluation is needed using blood gas evaluations, dosage and duration of barbiturate administration, this work indicates that there may be a protective effect in focal infarction in the primate afforded by barbiturate treatment. Barbiturate drugs may prove to be a desirable anesthetic for use in cerebral revascularization and other neurosurgical procedures.

Preliminary quantitative analyses of EEG, CBF, pCO_2 in acute ischemic and peri-ischemic zones, suggest that barbiturates retard the momentum of the ischemic insult but permitting gradual increase in $CMRO_2$ in peri-ischemic zones during the withdrawal period from the drug (biological half-life = 3-4 hours, pentobarbital). This delay brings O_2 demand into phase with collateral vascular bed reaction time (3-4 hrs. in these experiments).

Proposed Course: Additional work is planned to assess the changes in blood gases, circulation, and possible sites of action of the barbiturate during the next fiscal year. This work will be supplemented by investigations into the rate of (endogenous) revascularization. An experiment has been designed to distinguish hypertrophy from angiogenesis in the reactive vasoproliferation stages of ischemic infarction.

Further experiments to be done in collaboration with the Department of Neurosurgery, A. Einstein College of Medicine, will test the hypothesis that the addition safe time purchased by use of barbiturate anesthesia will permit effective neurosurgical intervention in the forms of emergency embolectomy and temporal-middle cerebral artery anastomotic bypass.

Keyword Descriptors: hypometabolic treatment, barbiturate anesthesia, ketamine analgesia, stroke model (primate)

Publication:

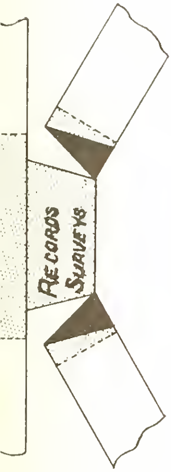
Moseley, J.I., Laurent, J.P., Molinari, G.F.: Barbiturate attenuation of the clinical course and pathological lesions in a primate stroke model. Neurology (in press)

W TO USE
ESE SEPARATORS

e one page for
ch separation.

lect appropriate
b, add further
entification if
sired, and cover
with scotch
pe.

t off and discard
l tabs except the
covered by tape.



ABBED SEPARATOR SHEET
rm HEW-69
-56)

ANNUAL REPORT
JULY 1, 1974 THROUGH JUNE 30, 1975
EPIDEMIOLOGY BRANCH
SECTION ON GENETICS IN EPIDEMIOLOGY
NATIONAL INSTITUTE OF NEUROLOGICAL AND COMMUNICATIVE DISORDERS AND STROKE

Introduction

During the past year the formal program of the Epidemiology Branch has been terminated with the exception of the program of the Section on Genetics in Epidemiology. Dr. Jacob Brody, Branch Chief, has been transferred to Intramural Research, NINCDS, and is now on detail to the Atomic Bomb Casualty Commission at Hiroshima, Japan. Dr. Roswell Eldridge is acting Branch Chief. Lon White, M.D. and George Nemo, Ph.D. and the laboratory facilities of the Epidemiology Branch have been transferred to the Laboratory of Central Nervous System Studies, Intramural Research. Responsibility for the Epidemiology Branch contracts outstanding has been transferred to William Weiss, Chief of the Office of Biometry, C&FR. The Section on Genetics in Epidemiology is to be transferred to Infectious Disease Branch, Intramural Research and will be retitled Section on Neurogenetics. An additional important function of the Section will be to proceed with recently initiated immunogenetic studies of diseases such as multiple sclerosis and amyotrophic lateral sclerosis where genetically determined immunologic abnormalities are suspected.

The program of the Section on Genetics for the past year has involved three principle areas:

I. Immunogenetic disease

II. Movement disorders

III. Hereditary CNS neoplasms

Among the more interesting accomplishments of the Section either directly or in collaboration with others are: (1) further definition of the biochemical abnormality in autosomal dominant torsion dystonia (see Part II, paragraph 1 below); (2) documentation of at least two hereditary forms of amyotrophic lateral sclerosis (see Part I, paragraph 2 below); and (3) development of a promising screening test for individuals at high risk for pheochromocytoma, such as those with the von Hippel-Lindau Syndrome (see Part III, paragraph 2 below).

I. Immunogenetic disease

A study designed to look at immunogenetic factors in multiple sclerosis is underway in collaboration with Multiple Sclerosis Societies in four geographically distinct areas. This work is prompted by the demonstration of an extraordinary association between one of the leukocyte determining antigens in the immune histocompatability complex and multiple sclerosis. Intensive serologic and immunogenetic studies will be carried out on approximately 20 families selected from these four regions.

Two forms of amyotrophic lateral sclerosis which are genetically determined have been distinguished over the past year by study of 34 documented cases in 14 families. The chief feature distinguishing them is their clinical course. One lasts between one and five years while the other uniformally lasts 10 years or more. In addition, there may be subtypes within these two groups based upon pathologic changes. HL-A typing has been done on 35 individuals in these families but results are not yet informative. Ninety-five at risk individuals are available for studies aimed at pre-clinical detection or earliest manifestations of this disease.

II. Movement disorders

Progress has been made in the biochemical characterization of the autosomal dominant form of torsion dystonia. Serum norepinephrine measured by techniques developed in Dr. Irwin Kopin's Laboratory of Clinical Science, NIMH is significantly elevated in many, but not all, of those affected with this disease so far studied. This observation supports those who have noted elevated serum dopamine beta hydroxylase in this disease. In contrast, in the autosomal recessive form, as seen in Jewish population, serum norepinephrine and dopamine beta hydroxylase are normal. Because of recent findings suggesting an association between catecholamine metabolism and purine metabolism studies in the latter area have been initiated.

An international conference dealing with the torsion dystonias has been organized with the assistance from members of this Section. It will be held in late May, 1975. Thirty-two papers will be given covering epidemiology, genetics, the relationship of dystonia to Parkinson's disease and Huntington's chorea, biochemistry, animal models, biofeedback therapy, drug therapy and neurosurgery. The Proceedings will appear as Volume XIII of the Advances in Neurology series.

Our preliminary study of the Gilles de la Tourette syndrome indicates that one form of this disorder is definitely familial and probably genetically determined. Previously unappreciated impulsivity involving aggression, sexuality and self mutilation suggest a relationship to the Lesch-Nyhan syndrome, an X-linked disorder of purine metabolism. Biochemical studies are underway in collaboration with members of the Laboratory of Clinical Science, NIMH, Dr. Jay Seegmiller and Dr. William Nyhan at the University of California at San Diego, and Dr. Melvin Van Woert, Department of Pharmacology, Mount Sinai Hospital, New York City.

III. Hereditary central nervous system neoplasms

Studies of nerve growth factor in hereditary central neurofibromatosis have shown elevation of this agent in preliminary studies. Additional studies await improvement in the assay for this factor in the medical genetics laboratory, Department of Medicine, Johns Hopkins Hospital.

The study of 50 individuals from nine families with von Hippel-Lindau syndrome indicates this may be a genetically homogeneous disorder with marked reaction in clinical expression. Morbidity and mortality result from the hemangioblastomas which may occur in the retina, posterior fossa of the brain, and spinal cord or pheochromocytoma and renal cell carcinoma. Each can be treated if observed in time. Indirect ophthalmoscopy is a simple and effective method for screening at risk family members for the first disorder. Serum norepinephrine can now be directly measured by our collaborators. A promising new screening procedure for pheochromocytoma is being evaluated in collaboration with members of the Laboratory of Clinical Science, NIMH. It is markedly elevated in two individuals in our series, one of whom had unilateral pheochromocytoma in the past. This suggests a screening for high risk individuals.

Progress has not been made on previously initiated studies of Parkinsonism, Huntington's chorea and myoclonic epilepsy because of manpower limitations in the Genetics Section and in laboratories of our collaborators. We have been following 19 families in which 37 are affected with one of the five different types of progressive myoclonus epilepsy with dementia. Over the past year at least four individuals from this registry have died and autopsy obtained on two. An intensive clinical and biochemical evaluation of selected families from this registry awaits adequate professional personnel in the laboratory of Dr. Anatole Dekaban, Developmental and Metabolic Branch.

A catalogue of the more than 500 specific neurogenetic traits already recognized is being prepared by the Section. Also, a directory of individuals at the National Institutes of Health with experience in genetic research has been prepared. Of the 74 individuals included in the present edition, five are from NINCDS.

Serial No. Z01 NS 00201-20 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam (ALS-PD)

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been transferred to IR and will be continued under Serial # Z01 NS 00201 - 20 ODIR

Serial No. Z01 NS 01103-12 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Neurological diseases other than ALS/PD on Guam

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 01319-09 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A search for autoimmune mechanisms in the pathogenesis of chronic neurological diseases by the use of peripheral lymphocytes

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 01487-08 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Genetic analysis of family data on Guam ALS and PD cases

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 01488-08 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Serological studies of common viruses in cases of multiple sclerosis (MS) and controls

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 01496-08 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Sequelae of CNS diseases in childhood and perinatal period

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 01597-07 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Neurological diseases in the Trust Territories and other Pacific areas

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 01605-07 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Phenothiazine-induced parkinsonism in white and Negro patients with nonorganic psychoses

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 01774-06 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Neurologic signs and symptoms associated with malabsorption

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 01777-06 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: ALS among non-Chamorros after residence on Guam

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 01832-05 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Serologic responses of multiple sclerosis patients and controls to a virus isolated from a multiple sclerosis case

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No Z01 NS 01833-05 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immune mechanisms in chronic and congenital viral infections

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 01835-05 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Epidemiologic and immunologic study of families of subacute sclerosing panencephalitis patients and families of matched controls

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 01837-05 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Multiple sclerosis distribution and patterns

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 01843-05 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Causes of death among siblings of MS and ALS patients

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 01844-05 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Amyotrophic lateral sclerosis in veterans

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 01917-04 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Twin study of multiple sclerosis: An epidemiologic inquiry

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 01920-04 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A study of pregnancies among nurses; high risk pregnancies with possible viruses exposure

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 01921-04 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A search for measles virus nucleic acid in cells of patients with multiple sclerosis (MS)

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 01922-04 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Search for a negative DNA strand in cells infected with the minute virus of mice (MVM)

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 01923-04 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Development of in vitro cellular immune tests for viral antigens

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 01924 05 E
1. Epidemiology Branch
2. Section on Genetics in Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biochemical and pharmacologic studies in torsion dystonia

Previous Serial Number: NDS (CF) - 71 E 1924

Principal Investigator: Roswell Eldridge, M.D.

Other Investigators: Michael Ziegler, M.D.
Raymond Lake, M.D.
Irwin Kopin, M.D.
William Horton, M.D.
William Kanter

Cooperating Units: Laboratory of Clinical Science
National Institute of Mental Health

Man Years:

Total: 3/5
Professional: 2/5
Other: 1/5

Project Description:

Objectives: Torsion dystonia consists of at least two hereditary conditions as well as an acquired form. Through appropriate biochemical investigation it should be possible to determine the precise biochemical defect (or abnormal gene product) in the hereditary forms of torsion dystonia. Appropriate treatment for each form should then be possible. A predictive test for use in those at risk might be possible.

Methods employed: Plasma has been collected from affected patients, quick frozen, and assayed for various catecholamine levels in the search for a specific enzyme abnormality. Double blind, crossover drug trials using promising agents such as Dantrolene Sodium (Eaton Laboratories) may be undertaken.

Major findings: Observations in 55 patients with torsion dystonia indicated that the enzyme dopamine beta hydroxylase (DBH), which governs the conversion of dopamine to noradrenalin, is present in abnormally high levels in individuals with the dominant form of torsion dystonia. Serum norepinephrine itself has been determined by a newly developed assay and found greatly elevated in some, but not all, with this form of dystonia.

Significance to biomedical research and the program of the Institute: Demonstration of the molecular lesion in one of the torsion dystonias would be a noteworthy achievement in the quest for inborn errors of metabolism. In addition, important information may develop regarding biochemistry and physiology of movement control. Specific treatment and pre-clinical detection useful in the genetic counseling of these families may be more immediate rewards. In addition, because of the recognized association of increased intelligence and the recessive form of dystonia, we stand to gain information regarding the development of intelligence. Pin-pointing the most effective treatment will afford immediate relief to those affected and may suggest the biochemical basis.

Proposed course: Continued biochemical and pharmacologic investigation of patients from the genetic registry. Addition of more sophisticated biochemical techniques such as that involving somatic cell hybrids with altered dopaminergic receptor function to the analysis of various tissues and evaluation of promising treatment regimens as they become available.

Honors and Awards: None

Publications: Eldridge, R.: The torsion dystonias: Literature review: Genetic and clinical studies. In the torsion dystonias (Dystonia Musculorum Deformans). Editor, Roswell Eldridge. Neurology, suppl. 20:11, part 2, pp. 1-78, 1970.

Chase, T.N.: Biochemical and pharmacologic studies of dystonia. In the torsion dystonias (Dystonia musculorum deformans). Editor, Roswell Eldridge. Neurology, suppl. 20:11, part 2, 1970.

Eldridge, R., Harlan, A., Cooper, I.S., and Riklan, M.: Superior intelligence in recessively inherited torsion dystonia. The Lancet, 65-67, 1970.

Wooten, F., Eldridge, R., Axelrod, J., and Stern, R.: Elevated plasma dopamine beta hydroxylase activity in autosomal dominant torsion dystonia. N. Eng. J. Med. 288:6, pp. 284-287, 1973.

Wooten, F., Eldridge, R., Gordon, E.K., and Axelrod, J.: Studies of catecholamine metabolism in two hereditary forms of dystonia. Trans. Amer. Neurol. Ass. vol. 98, 1973.

Eldridge, R., Kanter, W., and Koerber, T.: Levodopa in dystonia. The Lancet, II:7836, pp. 1027-1028, 1973.

Serial No. Z01 NS 01925 05 E
1. Epidemiology Branch
2. Section on Genetics in Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Intelligence in Israeli patients with torsion dystonia

Previous Serial Number: NDS (CF) 71 E 1925

Principal Investigator: Roswell Eldridge, M.D.

Other Investigators: Morris Gross, M.D.

Cooperating Units: Department of Education
Herbert H. Lehman College
Bronx, New York

Man Years:

Total: 3/10
Professional: 1/10
Other: 1/5

Project Description:

Objectives: The recessive form of torsion dystonia has recently been shown to be associated with increased intelligence. However, the series on which this information is based was small and individuals were scattered over a large area of northeastern part of the United States. In Israel, where we suspect there to be over 20 patients with the recessive form of torsion dystonia, a more homogeneous testing situation prevails so that results should be more meaningful. If this association is true the implications are dramatic so that confirmation to this Israeli study is clearly in order.

Methods employed: Bona fide cases of torsion dystonia in Israel would be selected by the principal investigator on the basis of personal examination. Psychometric data would be obtained in a retrospective manner through the Israeli Department of Education and similar data would be obtained for unaffected sibs. A comparison would then be made of the performance in these two groups.

Major findings: Although we have known for several years of the presence of over 20 cases of torsion dystonia in Israel we have not been able to arrange for visitation. Neurologists, psychologists, and educators in Israel have been alerted and indicated their interest in participating in this study.

Significance to biomedical research and the program of the Institute: The positive association between torsion dystonia and intelligence would suggest that the chemical abnormality producing dystonia may also enhance intellectual development. Elucidation of this basic chemical abnormality would suggest a method for enhancing intellectual development, particularly those from the retarded population.

Proposed course: Several weeks in Israel would be necessary to make the necessary home visits and arrange for the collection of appropriate psychometric data but travel funds have not been available for over the past 3 years.

Honors and Awards: None

Publications: Eldridge, R., Harlan, A., Cooper, I.S., and Riklan, M.: Superior intelligence in recessively inherited torsion dystonia. The Lancet, 1:7637, pp. 65-67, 1970.

Serial No. Z01 NS 01927 05 E
1. Epidemiology Branch
2. Section on Genetics in Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Report: Clinical, genetic, biochemical and pathologic studies in hereditary early onset acoustic neuroma

Previous Serial Number: NDS (CF) 71 E 1927

Principal Investigator: Roswell Eldridge, M.D.

Other Investigators: David Siggers, M.D.
David Rogers, M.D.

Cooperating Units: Department of Medicine
Johns Hopkins Hospital

Man Years:

Total: 2/5
Professional: 1/5
Other: 1/5

Project Description:

Objectives: To clarify the relationship of classical "peripheral" neurofibromatosis to early onset hereditary bilateral acoustic neuroma in selected clinical and laboratory techniques. To evaluate these findings as possible diagnostic marker for the former disease.

Methods employed: Obtain blood for determination of Nerve Growth Factor levels.

Major findings: Elevated nerve growth factor has been found in hereditary acoustic neuroma.

Significance to biomedical research and the program of the Institute: Through the study of nerve growth factor, it may be possible to define the relationship between hereditary bilateral acoustic neuroma and neurofibromatosis and also provide a genetic marker for hereditary acoustic neuroma. This latter information would be of great aid for purposes of genetic counseling and indicating those relatives that require maximum surveillance.

Proposed course: Proceed with investigations as outlined pending perfection of techniques at Johns Hopkins Hospital.

Honors and Awards: None

Publications: Allen, J., Eldridge, R., and Koerber, T.: Acoustic neuroma in the last months of pregnancy. Amer. J. Obst. Gyn. 119:4, pp. 516-520, June 1974.

Serial No. Z01 NS 01930 05 S
1. Epidemiology Branch
2. Section on Genetics in
Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Von Hippel-Lindau syndrome: Clinical, genetic and biochemical aspects

Previous Serial Number: NDS (CF) 71 E 1930

Principal Investigator: William Horton, M.D.

Other Investigators: Roswell Eldridge

Cooperating Units: Armed Forces Institute of Pathology
Johns Hopkins Hospital, Baltimore, Maryland
Columbia Presbyterian Medical Center, New York
University of Kansas Medical Center, Kansas
Laboratory of Clinical Science
National Institute of Mental Health

Man Years:

Total: 3/10
Professional: 1/5
Other: 1/10

Project Description:

Objectives: To review the clinical and pathologic findings in several families with von Hippel-Lindau syndrome.

Methods employed: Fifty individuals from 9 families manifesting the von Hippel-Lindau syndrome were evaluated by physical examination including indirect ophthalmoscopy and review of medical records and autopsy records.

Major findings: Analysis of this data combined with a review of 153 documented cases from the literature show that almost all of the morbidity and mortality of the syndrome results from hemagioblastoma of the eye; posterior fossa and spinal cord; pheochromocytoma and renal cell carcinoma. Since successful treatment is available for these lesions, early detection becomes of utmost importance and indirect ophthalmoscopy appears to be the easiest and most effective method of screening at risk family members. In addition serum norepinephrine determination may be valuable in detecting pre-clinical pheochromocytoma as evidenced in one case.

Significance to biomedical research and the program of the Institute: See "Major findings".

Proposed course: Currently 29 affected and 80 at risk members of these families are being followed to further evaluate the natural history of the disorder.

Honors and Awards: None

Publications: To be presented at the National Foundation Birth Defects Conference in Kansas City, MO, June 1, 1975.

Serial No. Z01 NS 01977 04 E
1. Epidemiology Branch
2. Section on Genetics in Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Selected genetic and clinical aspects of Huntington's Disease

Previous Serial Number: NDS (CF) 72 E 1977

Principal Investigator: Roswell Eldridge, M.D.

Other Investigators: William Horton, M.D.
Marjorie Guthrie
Thelma Koerber

Cooperating Units: Committee to Combat Huntington's Disease

Man Years:

Total: 2/5
Professional: 1/5
Other: 1/5

Project Description:

Objectives: To explore past and present attitudes of individuals with Huntington's disease and their relatives, and to determine the sources of information about the disease and family planning.

Methods employed: Historical research concerning views prevailing when this trait was first described; questionnaire survey of members of the Committee to Combat Huntington's Disease (CCHD).

Major findings: The chief concern is at present with the physical and mental aspects of this disease rather than the social, or genetic. For information, family members rely on CCHD more than on medical sources.

Significance to biomedical research and the program of the Institute: Lay groups such as CCHD can provide an efficient means for disseminating information and hope to patients and families confronted with distressing hereditary disease. In addition they can provide an atmosphere permitting those affected and at risk to come forward and discuss their attitudes and concerns.

Proposed course: Trace views toward Huntington's disease since the initial awareness in the United States. Add indepth analysis of patients, spouses, and at risk individuals regarding genetic counseling and screening procedures.

Honors and Awards: None

Publications: Eldridge, R., and Stern, R.: Huntington's Disease - Attitudes Toward Genetic Counseling. The American Society of Human Genetics, October 1973, Atlanta, Georgia.

Eldridge, R., and Stern, R.: Huntington's Disease - Attitudes Toward Genetic Counseling. J. Med. Gen. (In Press).

Serial No. Z01 NS 02045-02 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Epidemiology of polymyositis in children

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 02046 03 E
1. Epidemiology Branch
2. Section on Genetics in Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Clinical, genetic and biochemical study of the hereditary epilepsies

Previous Serial Number: NDS (CF) 73 E 2046

Principal Investigator: Roswell Eldridge, M.D.

Other Investigators: William Horton, M.D.
Salvador DeMoura, M.D.
Anatole Dekaban, M.D.

Cooperating Units: Department of Neurology, Columbia University
Developmental and Metabolic Neurology Branch, National Institute of Neurological Communicative Disorders and Stroke

Man Years:

Total: 1/20
Professional: 1/40
Other: 1/20

Project Description:

Objectives: To study the clinical, genetic and biochemical aspect of the hereditary epilepsies, such as the progressive myoclonic epilepsies, permitting their definition and specific prevention or treatment.

Methods employed: It has been possible to ascertain over 30 families with a diagnosis of progressive myoclonic epilepsy with dementia (PME D) in the United States. Examination of affected individuals and their relatives, documentation of family history and review of medical records provided the basis for an initial classification of these disorders. Laboratory studies including analysis of polyglucosan and mucopolysaccharides in various tissue are in progress as occasion permits.

Major findings: On the basis of the examination of thirty-seven affected individuals in nineteen families the following classification of PME D is suggested:

Type I (Lafora) Childhood onset, rapid progression, autosomal recessive inheritance, Lafora Bodies often present.

Type II childhood onset, slow progression, autosomal recessive inheritance, no Lafora bodies.

Type III (Unverricht-Lundborg) late adolescent onset, autosomal recessive inheritance, moderate or slow progression, occasional Lafora bodies.

Type IV (Hartung) late onset, variable progression, autosomal dominant inheritance.

Type V late adolescent onset, moderate to slow progression, autosomal dominant inheritance, neural hearing loss.

Significance to biomedical research and the program of the Institute: Distinction between the various diseases producing progressive myoclonic epilepsy and dementia is a pre-requisite for determining the specific metabolic defect in each disease. In a practical sense this classification should be of interest to clinicians faced with diagnosis of disorders characterized by myoclonic epilepsy and dementia. Documentation of inheritance pattern in these disorders permits improved genetic counseling for members of these families. In addition, such a study provides a model for the genetic study of seizure disorders.

Proposed course: To continue ascertainment of affected families and in course study of abnormal tissue by appropriate laboratories. Staff limitations in several research areas and lack of an easily accessible patient population has limited this project.

Honors and Awards: None

Publications: Stern, R., and Eldridge, R.: Clinical and genetic study of the progressive myoclonic epilepsies. J. Amer. Soc. Hum. Genet. pp. 76A, 1973.

Serial No. Z01 NS 02113 02 E
1. Epidemiology Branch
2. Section on Genetics in Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Side effects of L-DOPA on pre-pubital patients with dystonia

Previous Serial Number: NDS (GE) 74 E 2113

Principal Investigator: William Kanter

Other Investigators: Roswell Eldridge, M.D.
Lynn Loriaux, M.D

Cooperating Units: Reproduction Research Branch
National Institute of Child Health and
Human Development

Man Years:

Total: 3/10
Professional: 1/10
Other: 1/5

Project Description:

Objectives: Dopamine is in high concentration in the median eminence of the hypothalamus and probably is the principal mediator in the release of many hypothalamic releasing factors. An increase in growth hormone, FSH, LH, and a decrease in proactin have been demonstrated following the oral administration of L-DOPA. Theoretically, the administration of L-DOPA to pre-pubital patients might therefore interfere with normal growth and sexual maturation.

Children with dystonia represent probably the only group with pre-pubital patients in which L-DOPA has been used. We therefore intend to evaluate these patients in terms of the long term effects of L-DOPA on growth and sexual maturation.

Methods employed: Patients ascertained through previous studies of dystonia will be evaluated through a questionnaire regarding the effects of L-DOPA on growth through sexual development. Each matched dystonia patient never having been treated by L-DOPA and age matched patients will serve as controls. If the questionnaire suggests any significant differences in these groups, confirmatory biochemical studies will be performed.

Major findings: Results to date show 5 percent of dystonia patients surveyed noticed changes in sexual interest or development concurrent with levodopa treatment. We are initiating further communication with these individuals to assess the relationship between levodopa and changes noted.

Significance to biomedical research and the program of the Institute: Since the beneficial effects of L-DOPA in the treatment of dystonia are somewhat controversial, the finding of interference of normal development would support the argument that this drug should not be used at least not in this age group.

Proposed course: We are awaiting availability of suitable patients for NIH in patient study.

Honors and Awards: None

Publications: None

Serial No. Z01 NS 02114-02 E
1. Epidemiology Branch
2. Section on Genetics in Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The study of familial Parkinson's disease

Previous Serial Number: NDS (CF) 74 E 2114

Principal Investigator: William Horton, M.D.

Other Investigators: Roswell Eldridge, M.D.

Cooperating Units: None

Man Years:

Total: 1/20
Professional: 1/40
Other: 1/40

Project Description:

Objectives: Parkinson's disease is characterized clinically by a variety of extrapyramidal signs including muscular rigidity, tremor, and paucity of movement; biochemically by diminished dopamine in the substantia nigra and corpus striatum and pathologically by degenerative changes in the basal ganglia. Parkinson's disease has traditionally been classified by etiology into etiopathic postencephalitic and arteriosclerotic types. Recently several investigators have emphasized that a positive family history is often obtained in as many as 16 percent of the idiopathic type. Autosomal dominant, and more recently multifactorial inheritance, have been postulated to explain the familial nature of this disorder. We intend to examine this group in which a positive family history is obtained to see if there are any clinical and pathologic features which distinguich it from the more common sporadic disorder. In addition, genetic analysis of a large number of families may clarify the current understanding of the genetics. We also will compare this disorder to the Parkinsonism-Dementia syndrome endemic on Guam.

Methods employed: Families in which two or more members are affected by Parkinsonsim will be ascertained from selected medical centers. These families will be completely evaluated in terms of presence and extent of this disorder within family members. Further studies will be done when indicated.

Major findings: None

Significance to biomedical research and the program of the Institute: Delineation of the complete clinical spectrum of Parkinsonism may reveal several discrete disorders showing features of Parkinsonism but genetically and biochemically distinct This would allow more specificity in terms of treatment as well as more accuracy regarding diagnosis and prognosis

Proposed course: This project awaits additional staffing.

Honors and Awards: None

Publications: None

Serial No. Z01 NS 02115 02 E
1. Epidemiology Branch
2. Section on Genetics in Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Familial amyotrophic lateral sclerosis: Clinical, pathologic and genetic study

Previous Serial Number: NDS (CF) 74 E 2115

Principal Investigator: William Horton, M.D.

Other Investigators: Roswell Eldridge, M.D.
Paul Terasaki, Ph.D.

Cooperating Units: Department of Surgery
University of California

Man Years:

Total: 2/5
Professional: 1/5
Other: 1/5

Project Description:

Objectives: To characterize familial amyotrophic lateral sclerosis.

Methods employed: Thirty-four documented cases of familial amyotrophic lateral sclerosis from 14 families were evaluated by physical examination and review of medical records and autopsy material and records.

Major findings: Two forms can be differentiated on the basis of duration of course: one lasting one-five years and the other 10-25 years. In addition, there may be subtypes within groups separated by pathologic findings: the presence or absence of degenerative changes in the posterior columes, Clarke's column, and spinocerebellar tracts in addition to changes classically found in the anterior horn cells and corticospinal tracts. HL-A typing was done on 35 individuals including five who were affected, but results were uninformative.

Significance to biomedical research and the program of the Institute: See "Major findings".

Proposed course: Ninety-five at risk individuals are being followed and would potentially represent a population which could be studied in terms of pre-clinical and early manifestations of the disorder.

Honors and Awards: None

Publications: To be presented at the American Academy of Neurology Meetings to be held in Bal Harbour, FL on May 1, 1975.

Serial No. Z01 NS 02116-01 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Nucleic acid studies on selected oncornaviruses

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 02117-01 E
1. Epidemiology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Search for biochemical evidence of virus involvement in chronic neurological diseases

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued due to the departure of the senior investigator.

Serial No. Z01 NS 02167-01 E
1. Epidemiology Branch
2. Section on Genetics in Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunogenetic studies in multiple sclerosis families

Previous Serial Number: None

Principal Investigators: Roswell Eldridge, M.D.

Other Investigators: John Sever, M.D.
Paul Terasaki, M.D.
George Schumacker, M.D.
Bernice Schacter, Ph.D.

Cooperating Units: University of Vermont School of Medicine
Johns Hopkins Hospital
Multiple Sclerosis Societies of Kansas City,
Witchita, Milwaukee and Washington, DC

Man Years:

Total: 1/10
Professional: 1/20
Other: 1/20

Project Description:

Objectives: In approximately 8 percent of multiple sclerosis cases there is a positive family history. Recently there has been demonstration of an unusual association of several of the HL-A antigens with multiple sclerosis. Even more striking is the association with antigen of the mixed leukocyte culture system (MLC). A preliminary, unpublished report by Milton Alter from Minnesota suggests there may be linkage between the HL-A system and a gene or genes predisposing to multiple sclerosis. Because his study was done in a small number of families in a prescribed geographic area and did not include evaluation of MLC system a more comprehensive study has been launched. In addition to the evaluation of Dr. Alter's preliminary finding the more general role of genetic factors in multiple sclerosis will be explored.

Methods employed: Cases of MS will be identified in which the affected patient has at least one living first degree relative who also has MS (preferably parent and offspring and 3 or more with MS). Twenty to thirty such families will be identified through the above cooperating units. Affected individuals as well as their siblings and parents will be typed for HL-A antigen, serological determinants and lymphocyte determinants, (MLC). Specimens will be collected and shipped on the same day by air express to Dr. Paul Terasaki. Other typing will be added as possible.

Blood specimens will be collected as a 40 aa aliquot utilizing preservative-free heparin. Both the HL-A and MLC tests will be performed with the same heparin. The shipment labels must clearly indicate that MLC determinations be sent to the IDB, NINCDS when completed.

An additional 10 cc serum specimen must be obtained, and will be used for viral serology studies. Serum is preferred to heparinized blood for serological testing, and so must be collected as a separate specimen.

It will be extremely important to document the diagnosis of MS in all cases which are to be considered for study. For purposes of this study it will not be adequate to accept the referring diagnosis of MS. Instead it will be necessary to adhere to a uniform set of criteria for the diagnosis of MS, and of potential study cases.

Pal control family will also be studied in essentially the same way.

Major findings: None

Significance to biomedical research and the program of the Institute: Etiology in MS remains obscure. Because of the familial concentration a genetic predisposition is likely. The above study is designed to evaluate the role of immunogenetic factors in such predisposition.

Proposed course: Families are being ascertained through the various MS chapters. If staffing requirements permit, initiation of the study will commence in the summer of 1975.

Honors and Awards: None

Publications: None

Serial No. Z01 NS 02168-01 E
1. Epidemiology Branch
2. Section on Genetics in Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

oject Title: Clinical, genetic and biochemical observations in families with Gilles de la Tourette Syndrome (GTS)

evious Serial Number: None

incipal Investigator: Roswell Eldridge, M.D.

her Investigators:
Richard Sweet, M.D.
Arthur Shapiro, M.D.
Michael Ziegler, M.D.
Raymond Lake, M.D.
Jay Seegmiller, M.D.
Melvin van Woert, M.D.

operating Units:
Cornell Medical School
New York University
Laboratory of Clinical Science
National Institute of Mental Health
Department of Medicine
University of California
Department of Pharmacology
Mount Sinai Hospital

n Years:

Total: 1
Professional: 1/4
Other: 3/4

oject Description:

Objectives: The Tourette syndrome has been considered a relatively rare sorder. It is characterized by tics and other jerky movements plus un- ntrollable utterances coming on in childhood and following an irregular t generally progressive course through adolescence. In the past it was nsidered by most to be functional in origin. But the specific response of mptoms in GTS to Haloperidol together with absence of any consistent under- ing psychopathology suggest there is an organic basis to the symptomatology. though family history is said to be negative by most evaluating this estion there are four reports appearing since 1973 of parent and offspring fected.

In view of these developments a comprehensive evaluation of clinical and genetic aspects of this syndrome is warranted. Through appropriate biochemical studies a definition of the underlying metabolic abnormality will be attempted.

Methods employed: Initial families for evaluation were selected through the Tourette Association, a lay, patient organization headquartered in New York. During a one day clinic in New York City, genealogic, medical and neurologic history was obtained on all family members and those present were examined. Blood was obtained for catecholamine studies. Subsequently other studies have been undertaken regarding purine metabolism.

Major findings: Clinical points to emerge from the study which are not generally recognized are: uniform age of onset with most in the study group being 3 to 5½ years of age; the rather low maintenance dose of Haloperidol required in that group of patients benefitting from this drug; decrease in symptoms in many older patients; and the presence of socially unacceptable behavior in the areas of sexuality, aggression or self mutilation (or presence of powerful impulses in these same areas) in the majority of those affected.

A strong familial concentration is noted. Most families are Jewish.

Biochemical points to emerge are the absence of changes in dopamine beta hydroxylase, serum norepinephrine and monoamine oxidase. Preliminary studies of HGPRT levels suggest there may be some deficiency in this enzyme involved in uric acid metabolism.

Significance to biomedical research and the program of the Institute: Findings suggest there is a genetic form of GTS. Behavior patterns are similar to features of the Lesch-Nyhan syndrome, a recognized, x-linked disorder of purine metabolism. As the finding of normal catecholamine and questionable changes in purine metabolism suggest the latter area may indeed reveal the underlying defect in this disorder of movement and behavior.

Proposed course: A second clinical and genetic study should be undertaken to see if these same kind of familial concentration and a typical behavior is seen in a different sample. Expansion of catecholamine studies to include those involving the central nervous system and spinal fluid would be desirable. Studies of purine metabolism are underway in collaboration with groups at NIH, New York City and San Diego. Israel, where 50 cases have recently been reported, would be a fruitful area for additional collaboration.

Honors and Awards: None

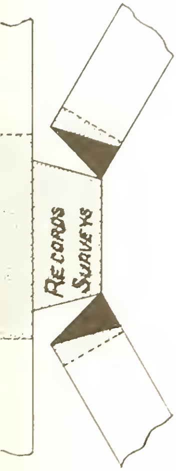
Publications: To be presented at the American Academy of Neurology meeting, Bal Harbour, Florida, May 1975.

W TO USE
HESE SEPARATORS

e one page for
ch separation.

lect appropriate
b, add further
entification if
sired, and cover
with scotch
pe.

t off and discard
l tabs except the
e covered by tape.



ABBED SEPARATOR SHEET
orm HEW-69
3-56)

ANNUAL REPORT
For Period July 1, 1974 through June 30, 1975
Office of Biometry
Collaborative and Field Research Program, National Institute
of Neurological and Communicative Disorders and Stroke

The Office of Biometry is in the Collaborative and Field Research Program, NINCDS, under the direction of the Associate Director, C&FR.

Its program responsibilities include surveys of neurological diseases and communicative disorders. It is responsible for the design, pretesting, and accomplishment of such surveys - whether under contract, in-house, or in cooperation with other government agencies - to obtain disease statistics for NINCDS program planning, and for the establishment of research priorities. It will establish a current, centralized, disease statistics data repository in NINCDS for providing disease statistics reports.

The Office of Biometry is responsible for monitoring existing research contracts in neuroepidemiology, and for developing a new program in that area.

The Office of Biometry is a central resource to NINCDS for service in statistics, mathematics, computer technology, systems analysis and data processing. Its services range from brief consultations to participation as co-investigators on major projects.

The Office of Biometry carries out research in mathematical statistics for current and anticipated use in NINCDS programs. It also provides consultation and service to other organizations undertaking studies in the areas of NINCDS interest.

The Office of Biometry consists of the Office of the Chief, the Section on Mathematical Statistics, the Section on Disease Statistics Surveys, and the Section on Systems Design and Data Processing.

The report on the projects and services performed by the Office of Biometry is listed by major program area, and followed by brief descriptions of Research, Miscellaneous Activities, Publications, and Future Plans.

OFFICE OF THE DIRECTOR, NINCDS

1. Fact Finding Report Relative to Information Systems for NINCDS

Principal Investigators: Dr. L. Fox, William Weiss, Sebastiano A. Sciabbarrasi, Bernard H. Kroll

The purpose of this investigation was to compare all NIH systems which are used to gather and report on administrative and research elements of the Institutes in order to evaluate their use. The important areas of grants, contracts, personnel and budget were specifically examined to ascertain their use of systems and their needs for information.

The investigation included other agency systems as well as some commercial applications.

A report to the Director was prepared, which summarized the study findings and recommended a design for an in-depth study to prepare for the development of a program information system.

2. Design and Implementation of a Program Information System

Principal Investigators: William Weiss and Bernard H. Kroll

The concept of a program information system has been developed in anticipation of the reorganization of NINCDS, with a recognition of the future increased emphasis on program planning and evaluation within the Institute and with the expressed need by the Director and his planning staff for rapid, detailed, and flexible means of obtaining information about the research program and the general administration of NINCDS.

A preliminary draft of a contract project plan and request for proposals (RFP) is in preparation. It is anticipated that DHEW Set-Aside Evaluation Funds will be requested in support of the development of the Program Information System. This will enable the Institute to obtain the professional assistance needed to develop the system specifications and the additional programming support for the entire system.

3. Annual Automatic Data Processing (ADP) Report

Principal Investigators; Dr. Donald Tower, Bernard H. Kroll and
Sebastiano A. Sciabbarrasi

The purpose of the annual study is to obtain and report all needs for automatic data processing (ADP) systems, software and services of the Branches and Laboratories of the Institute. It also estimates the financial requirements for these requests and presents them as an integrated package to higher authority for review and concurrence.

It permits an orderly development of ADP needs each year within the funds available for expenditure, and consistent with the research plans of the Institute.

The system also includes reports on existing systems and software and meets the OMB requirements for reporting of machines, terminals and personal data systems.

COLLABORATIVE AND FIELD RESEARCH PROGRAM

Office of Biometry

1. Disease Statistics Survey Program

Present research contracts include:

Head and Spinal Cord Injury Survey
Intracranial Neoplasms Survey
Multiple Sclerosis Survey

The Office of Biometry organized a multi-disciplinary advisory committee to develop a design for a survey of stroke. During a series of meetings, the committee developed a set of recommendations which were incorporated in a Request For Proposal which has been issued for a survey of stroke.

The DHEW Set-Aside Evaluation Fund has allocated $300,000 to support methodological research in the stroke survey.

The Office of Biometry is collaborating with the National Center For Health Statistics to design a questionnaire on stroke to be incorporated in the 1977 Health Interview Survey in order to provide additional data relating to stroke.

The Intracranial Neoplasms Survey has received OMB clearance; the other two survey OMB clearance documents are in the process of review.

The pilot study for the Intracranial Neoplasms Survey has begun, in order to test potential solutions to a large number of methodological problems. The pilot studies for the other disorder surveys will begin immediately after OMB clearance.

This major program in incidence, prevalence, and economic costs of disease by NINCDS has apparently created a considerable degree of interest on the part of the DHEW Planning and Evaluation Office. This interest is providing an impetus for NIH to coordinate the dissemination of information on surveys among the sister Institutes.

The DHEW Planning and Evaluation Office is also emphasizing the need to ensure the comparability of economic cost data across disease lines. The Office of Biometry's initial plans were to provide comparability across disease lines within the diseases it surveys by coordinating the efforts of the economic staffers and consultants of the various contractors. An initial meeting of these economists, arranged by the Office of Biometry while the pilot studies were in development, created a consensus of the direct and indirect costs to be measured, though the contractors were encouraged to vary the methods for obtaining cost data. These economists will meet again after the pilot studies have been completed, to agree on a consistent approach to cost evaluation.

2. Study of Multiple Sclerosis in the Shetland and Orkney Islands

Principal Investigator: Dr. David Poskanzer

The Office of Biometry is assisting in the study by providing forms design, data processing and computer services.

The forms have been designed, tested, and are in use within the Islands. A preliminary group of coded forms has been processed, and files created, and tabulations have been sent to the principal investigator and his staff for review and evaluation for possible additional forms changes or coding problems.

Additional tabulations and analyses are expected as new data are provided.

3. Development of Justification for a National Pain Data Center

The Office of Biometry has studied the concept, function and potential utilization of a national data center for research on pain. Frequent discussions on this subject have been held with Dr. C.L. Li, Intramural Program, who strongly supports the general concepts.

Chronic pain is a disabling condition which constitutes a serious national health and economic problem. Its cost in human suffering is incalculable. Diagnosis and treatment of chronic pain are frequently very difficult, if not impossible. Millions of patients, suffering both physically and financially, are not obtaining the relief they deserve. The deficiencies of treatment of chronic pain are due mainly to a lack of understanding of the precise mechanism and physiopathology of chronic pain and its psychological and sociological complications; the lack of a sound concept of the management of chronic pain and the inadequate use of current knowledge and techniques available to the care of patients; and the lack of a well-organized national effort in coping with the pain problem. Common to all of these factors has been the paucity of communication among investigators, clinicians and public health workers. The lack of a data pool on pain, which could serve as the informational basis for scientific and clinical communications, is one of the causes of this problem. The development of a national pain data center to assume the function of systematic planning, collection, processing and retrieval of nationwide research and clinical data concerning pain is of primary importance in this field. It will provide data support to research investigators and clinicians specializing in problems relating to pain; serve the public need by supplying health statistics, clinical and therapeutical data relating to pain; develop health statistics and epidemiological studies of pain, and provide a means for conducting statistical research on the quantitative methodology in research on pain.

4. Quantitative Testing and its Usefulness in Evaluating Therapy

Principal Investigator: D. Sadowsky

Quantitative evaluation of the functional capacity of patients undergoing therapeutic trials is becoming more useful to researchers in neurology, rheumatism, mental health, cancer, etc. The Office of Biometry is formulating a battery of Clinical Quantitative Tests (CQT) which will (1) be standardized (conducted the same way using the same equipment, checked for consistency, etc.); (2) have established normals (to truly measure disabilities, to produce "profiles" of disease, etc.); (3) have the proper selection of tests (comprehensive for the disease, no redundancy, motivational to patient, etc.); (4) tested for validity and reliability (measures function well, measurements are reproducible, etc.); and (5) have an official NIH form for the data to be included in the patient's Clinical Center medical records.

After acceptance of the battery of CQT as an official NIH form, it is proposed to use it to (1) train examiners to administer the tests; (2) administer the tests to a large enough group of matched (on age and sex) normal subjects to obtain normal values; and (3) have the battery available for NINCDS, NIMH, NCI, etc. researchers to use for objectively evaluating the effectiveness of new drugs, therapy, and surgical techniques which improve the performance of the patients.

This proposal has been presented to some of the NINCDS scientists (Drs. J. Van Buren, A. Ommaya, K. Engel, T. Chase and his group, and therapists) for their comments and criticisms. The plan and the form have been most favorably received by them; they have reviewed the plan and the form and made suggestions for improvement; and they have offered assistance in obtaining both the established normals and the diseased patient data.

5. NEUROMED

Principal Investigators: Robert Richter, Alan Talbert

The purpose of this project was to develop a computer system to enhance, expand and simplify the use of multivariate analysis methods, in such a way that individual statisticians and data analysts could use computers more intensively and effectively in a broad range of consulting activities.

Dr. Peter Shaughnessy, formerly of the staff of the Office of Biometry, was responsible for, and made a significant contribution to, the design, scope, and specifications for the system prior to his departure.

The file handling input procedures and program for the system were designed and written, followed by the design and programming of the NEUROMED operating systems and its outputs.

The system was completed this year and is in its implementation phase.

A presentation of the system was made in April, 1975 to the Eleventh Symposium on Biomathematics and Computer Science in the Life Sciences at Houston, Texas.

A paper has been submitted for publication to the data processing journal "Software Practice and Experiences".

6. Computer Applications

Tail-Sensitive Normality Testing Program

Principal Investigator: Alan J. Talbert

A computer program for testing normality of frequency distributions has been written and is ready for use. The user can specify the number of middle (non-tail) intervals. In an effort to optimize sensitivity of the tests (Chi-square) in the tails, the program has been designed to permit the user to specify a proportion of the total frequency to be included in the tails. The program will operate in batch or TSO mode and can serve as an introduction to the use of TSO for Office of Biometry statisticians. It was designed for use with the Minimal Brain Dysfunction Study, but can be quite general in its application.

7. Consultation and Data Processing - NINCDS Computer Projects

Principal Investigators: (see below)

One of the Section's functions is the consultation on computer application and methods, and, when requested, the programming of small volume studies for professional staff of the Institute. Examples of these studies are as follows:

- Research Control Data System - NINCDS Contracts Office
- Gilles de la Tourette Study - Dr. Eldridge
- Natural History of Parkinson's Dementia on Guam - Epidemiology Branch Staff
- Genetic Interest Survey - Dr. Eldridge
- Von-Hippel - Lindau Syndrome Study - Dr. William Horton
- Study of Familial Anterior - Lateral Sclerosis (ALS) - Dr. William Horton
- Paper Tape for Generating Sounds for Hearing Tests - Dr. L. Elliott

8. Additional Activities

Staff members have served on a number of site visit teams for various Institutes provided consultation to a DRG Study Section with regard to a grant application, and lectured to professional and other student groups on subjects in systems design, computer programming, or software use. Also a staff member has, using NLM, Medline and the NIH library, researched available publications for studies of incidence and prevalence for a number of neurological diseases. Publication of these lists is contemplated.

Perinatal Research Branch

1. Minimal Brain Dysfunction Study

Principal Investigators: Drs. P. Nichols and T. C. Chen

The purpose of this study is to describe MBD children in the CPP population with descriptors selected from four categories, namely, cognition and perception, learning, behavior, and mild physical defects; and to investigate the relationship between the incidence of MBD and a number of precursors and antecedent events in the Collaborative Perinatal Project. During the past year (1) a series of statistical studies has been conducted to develop some statistical criteria which can be used to describe MBD in a parsimonious way. It has been observed from these analyses that very consistent and statistically demonstrable correlation and factor analytical results existed in all sex-race groups, indicating that some 4 or 5 factors can be extracted from the 25 descriptors selected originally, and that the scores of these factors evaluated for each child can provide an appropriate basis for the classification of MBD's; (2) Since the 7-year data file which contains the major sources of data for the MBD study has been updated due to the addition of new data, the MBD study file was accordingly revised and a new data file was created, and is the final version of the file; (3) correlation and factor analysis, estimation of factor scores, and examination of the distributions of factor scores for defining MBD will be carried out with the revised file to generate a basis for a definitive classification of MBD children. A part of the statistical analysis has been completed with white-male data; (4) the feasibility of using an alternative statistical approach such as dichotomization of descriptor data and cluster analysis with binary information has been studied. The possibility of developing a computer program with an efficient algorithm for cluster analysis of an extremely large group of data is under study by one of our statisticians.

2. Birthweight-Gestation Study

Principal Investigators: Drs. Janet Hardy and E. David Mellits,
Johns Hopkins

The Office of Biometry has participated in preliminary analyses of data for a study of prematurity based on the Collaborative Perinatal Project data. The first phase, to study the relationship between birthweight and gestation, to evaluate the effect of other measures such as placental weight, and to determine whether their addition to the combination will more precisely define prematurity, is almost complete. Multiple regression analysis and analysis of covariance are being used to analyze the relationships. Special computer programs had to be written for the analysis.

New indices of risk, based on combinations of these 'prematurity' variables, will be created and tested to determine their utility in assessing the etiology of prematurity.

3. Cerebral Palsy Study

Principal Investigators: Drs. Karin B. Nelson and
Jonas H. Ellenberg

The Office of Biometry, in collaboration with PRB staff has made significant advances toward completion of the analysis of these study data of the Collaborative Perinatal Project. First, the cerebral palsy tape has been created and tested, and identifies all children with cerebral palsy diagnoses in the Collaborative Perinatal Project in much greater detail and with a higher degree of accuracy (through extensive chart review by PRB personnel) than heretofore available. Second, a new extensive pediatric variable file has been created and tested which takes special account of early neurological findings. The file is well documented and reconciles, where possible, inconsistencies in form revision and other sources. Finally, the first phase statistical computer program to screen antecedent obstetric variables and early clinical manifestations with regard to their association with cerebral palsy diagnoses has been written and tested. It is expected that we will complete the screen before the end of this fiscal year.

4. Convulsive Disorders Data Analysis

Principal Investigators: Drs. Karin B. Nelson and
Jonas H. Ellenberg

The analysis involved in the convulsive disorders study during the current fiscal year overlapped with the Cerebral Palsy Study, described elsewhere in this report. In the initial data analysis phase, we have found it efficient to take similar approaches in the two studies. After the first phase data processing request is completed, the two studies will diverge in their goals and required analyses.

5. Microcephaly and Macrocephaly Study

Principal Investigators: Drs. Karin B. Nelson, and
Jonas H. Ellenberg

The objectives of the study on micro and macrocephalic children in the Collaborative Perinatal Project are twofold. First, to describe the deviations from normal head size growth in a longitudinal fashion rather than at single points in time, and second, to examine the prognosis of children with abnormal growth patterns. A procedure was devised to compare each child's head size at the several time points of measurement during its first seven years of life, to the respective study norms, adjusting for age, sex, race, institution and gestational age. Deviant children have now been identified, and planning is under-way for a concise methodology to be used in describing the growth pattern of these children.

6. Congenital Malformations Study

Principal Investigators: Drs. N.C. Myrianthopoulos, A.F. Naylor,
Mr. D. Rubinstein.

The Office of Biometry has participated in the planning and review of Congenital Malformations Studies. It provided statistical support in the analysis, evaluation of results, and publication, of a study on the relationship of blood types of mothers and children with the incidence of malformations in the latter.

7. Visual Abnormality Study

Principal Investigators: Dr. R. Feinberg and E. Jackson

The Office of Biometry has participated in designing the study plan and the detailed tabulation specifications.

Preliminary investigations are underway to determine the extent of various visual problems reported, the patterns of use of the special seven-year examinations (that is, the visual screening and ophthalmology consultant's examinations), and the proper source of various types of information. The ophthalmology consultant's reports have been reviewed and coded for use in the data analysis.

An analysis of the relationship between a large number of demographic, obstetric, and pediatric variables and various visual abnormalities is underway; the first half has been tabulated and reviewed, and the second half is being tabulated. Further studies in additional depth will be made of those variables which appear important.

8. Communicative Disorders Study

Principal Investigators: Dr. P.J. LaBenz, Ms. J.G. Oliver,
Mr. D. Rubinstein

The Office of Biometry provides an assistant project officer for the Speech, Language and Hearing Deficits contract study, who monitors the contract performance and advises on appropriate statistical methods, and interpretation of the results. Activities include the formulation of statistical criteria of data quality, the analysis of SLH quality control data, and the planning of their publication.

9. Study of the 7-Year Socio-Economic Index

Principal Investigator: Dr. P.L. Nichols

Mr. Rubinstein participated in the planning of the data displays and analyses related to the 7-year socio-economic index and to the socio-economic index obtained at registration. The analysis will consist of (a) establishment of the distribution of the index in various subgroups, (b) correlations with other factors, and (c) appropriate cross tabulations.

10. "The First Year of Life", a second volume continuing the presentation of the basic data of the CPP.

Principal Investigators: Dr. J. Hardy, Dr. S. Drage and E. Jackson

The Office of Biometry is participating in the preparation of the book. The effort includes data analysis, the procurement of computerized graphics, and the preparation and review of the text.

Office of the Associate Director for Extramural Programs

1. Otolaryngology Manpower Study

Principal Investigators: The Manpower Committee of the American Council on Otolaryngology and Office of Biometry Staff

The purpose of this study is to identify and describe the present otolaryngological manpower, estimate future needs, and recommend policy for training and development of these specialists in the 1980's. This project was contracted by the Extramural Program and Bernard H. Kroll is the contract project officer.

In the past two years, surveys were made of the specialty in clinical practice, and research and teaching (residency training).

The final report was submitted to the Extramural Program Director prior to the end of the fiscal year and plans exist to publish this product as a monograph together with the results of similar studies for other specialties which were completed at the same time.

All of the systems work was done by Bernard H. Kroll and Sebastiano A. Sciabbarrasi as part of the Manpower Committee activity. The extensive amount of data processing and programming to produce all the descriptive and analytical tables were accomplished by Sylvia Edelstein and Barbara Nichols.

2. Cochlear Implants Research Program of NINCDS Auditory Implant Research Centers

Principal Investigators: Drs. Murray Goldstein and
Lois Elliot

Dr. Jonas H. Ellenberg participated in a workshop on cochlear implants which attempted to answer questions related to the evaluation and further development of implants as an approach to the problem of hearing loss. He also participated in the initial staff review of the workshop and drafting of an NINCDS program announcement for a targeted research program of NINCDS Auditory Implant Research Centers (AIRC).

Intramural Research Program

1. A Study of Pain-Associated Reactions

Principal Investigators: Drs. C. L. Li and T.C. Chen

The purpose of this study was to investigate the association of physiological, hematological and blood chemistry reactions with pain in human subjects. The knowledge obtained from this study can be useful in broadening the understanding of pain, and providing a better basis for the measurement of the response of pain. The present study was planned to include experimental and clinical pain. Preliminary study involved a statistical investigation of the reactions with laboratory-induced pain by stimulating the left trigeminal nerve electrically near the supraorbital branch. Experimental data were obtained from 14 normal, healthy college students. Their physiological measurements (such as EEG, EKG, respiratory movement, skin temperature, blood pressure, pulse rate) and blood samples were taken before, during, and after pain induction. The blood samples were analysed for the hematological and serum chemistry values. Data analysis, including parametric and non-parametric two-sample tests, have been carried out to seek possible changes in hematological and serum chemistry parameters due to pain. Further analyses will be conducted to study the interrelationship of these parameters, and their changes, with pain.

2. Acupuncture Analgesia: A Study of Clinical Pain

Principal Investigators: Drs. C.L. Li, H. Lansdell, T.C. Chen

The Office of Biometry is participating in a study of the analgesic effect of acupuncture on trigeminal neuralgia -a pathological pain known to be associated with one particular nerve. The general plan of this study has been devised, but several detail problems remain to be solved. In the statistical portion of the collaboration an experimental plan for allocating patients to treatment groups in order to control the sex and age of the test subjects has been developed. The Melzack and Torgenson method of determining and rating subjective response to pain has been studied for its feasibility and significance to this study. The problems defining acupuncture treatment, selecting an appropriate placebo-acupuncture, and designing a two-stage experiment for the clinical trials have been considered.

3. Acupuncture and Hypnosis: Effect on Induced Pain

Principal Investigators: Drs. C.L. Li, H. Lansdell, D. Ahlberg
T.C. Chen, et al.

The purpose of this study was to investigate the effect of acupuncture analgesia, and to compare it with the analgesiac effect of hypnosis. Normal volunteers were selected to receive electrically-pulsed forehead pain before, during, and after acupuncture, placebo-acupuncture and hypnosis. Pain threshold was measured at three pain sensation levels. Other measurements taken involved physiology, hematology and blood chemistry. Consultation and support were provided in a comprehensive statistical investigation and analysis of experimental data by such techniques as analysis of variance, multiple analysis of variance, Hotelling's T^2 or t-test, etc., and assistance was also provided in the preparation and review of the study report for publication. The statistical result did not indicate strong evidence of an acupuncture effect on experimentally induced pain.

Laboratory of Central Nervous System Studies

1. A Study of Acute and Chronic Airway Disease

Principal Investigators: Dr. P. Brown and D. Sadowsky

Values for the measurements of vital capacity for 182 normal controls and 207 lung diseased (asthma, bronchitis, emphysema, etc.) patients were used to (1) derive formulas for predicting Forced Vital Capacity (FVC) and one-second Forced Expiratory Volume (FEV_1) from age, height, predicted FVC and FEV, etc.; (2) obtain predicted FVC and FEV_1 by sex and age; (4) obtain graphs of %FVC and $\%FEV_1$ by age including plots of normal controls, patients, description (or prediction) lines, and 95% confidence belts; and (5) compare normal controls to patients by age, height, FVC, FEV_1, %FVC, $\%FEV_1$, etc. All these analyses were accomplished by use of linear and multiple regressions, t-tests, analysis of variance studies, regression analyses, covariance analyses, graphing, etc. This material was presented as Obstructive Lung Disease in Micronesia by Brown, Sadowsky, and Gajdusek at the 48th Annual Meeting of the American Epidemiological Society in Los Angeles, April 4, 1975, and will be prepared for publication.

Laboratory of Neuropharmacology

1. A Study of L-Dopa and Other Drugs for Huntington's and Parkinson's Patients

Principal Investigators: Drs. I. Shoulson and Kartzinel

Eleven patients with Huntington's and Parkinson's Disease were administered Prolactin Carbi-dopa, and Piribedil after which their hormonal drug levels were tested. Data from these tests were analysed by applying (1) multivariate statistical methods to determine differences between patients, drugs, and times of hormonal tests and (2) t-tests to determine specific differences at various time points.

2. An L-Dopa Study

Principal Investigator: Dr. I. Shoulson

Seven Huntington's and Parkinson's patients were administered various dosages of L-dopa at various times during the day. Then, at 30 minute intervals, clinical and plasma drug levels were tested. The data from these tests were analyzed by multiple analyses of variance and graphed for each patient. This will be a continuing project.

Laboratory of Neuropathology and Neuroanatomical Sciences

1. Morphological Studies of CNS Demyelination and Remyelination in Model Systems.

Principal Investigators: Drs. H. Webster and T. Tabira

Double blind tests were conducted by injecting various substances (CSF sera from patients with or without demyelinating disease, rabbit sera, etc.) into the optic nerve of tadpoles. The nerves were removed in order to count the lesions created in them by the injected substances. All experiments were conducted using randomized and double blind slide selection. The data from these lesion counts were tested for differences between cases and controls, inoculated and non-inoculated sides, various substances, etc., by use of the Kolmogorov-Smirnov Two-Sample Test, and other tests.

Infectious Diseases Branch

1. Normative Hematological Limits of Cebus Monkeys

Principal Investigators: Drs. A. Palmer and T.C. Chen

The purpose of this study was to determine normative limits of a number of hematological parameters of Cebus monkeys, using 159 females and 51 males selected at random. Statistical collaboration included: (1) conduct of a pilot statistical study to determine various sources of experimental variation, (2) collaboration in planning the main study, designing the experiment and determining the sample size to generate adequate data information, (3) development of statistical formulas for evaluating component variances for the hematological measurements with frequent missing data, (4) definition of statistical normalcy and the carrying out of computations for means, standard deviations, standard errors by means of transformation of variables, analysis of variance, component variance analysis, etc.; hence, deriving normative limits (in terms of statistical confidence and tolerance) for each hematological measurement, (5) preparation of a statistical report exemplifying a complete concept and methodology that is appropriate for the other similar types of research.

2. Herpesvirus Induction of Cervical Cancer in Cebus Monkeys

Principal Investigators: Dr. William T. London, Andy Nahmias
(Emory Univ.) and Dr. Jonas H. Ellenberg,

Several studies have indicated that herpesvirus (HSV) type 2 may be etiologically related to human cervical cancer. This study is investigating the possible causal relationship between genital herpes and cervical cancer by attempting to induce cervical cancer in monkeys inoculated genitally with the virus. The statistical design of the study was produced by the Office of Biometry in FY 72. Continual monitoring of the statistical aspects of the study design have been necessary due to the development of heretofore unknown conditions relating to the interaction of the HSV-2 virus in the Cebus model. A manuscript has appeared in Cancer Research, relating the methodological approach to the problem. A member of the Office of Biometry is a co-author.

3. Investigation of Incubation Period for Subacute Sclerosing Panencephalitis

Principal Investigators: Dr. John L. Sever, Eudora L. Beadle,
and Dr. Jonas H. Ellenberg

Subacute sclerosing panencephalitis (SSPE) is a slowly progressive brain disease which is uniformly fatal. This study involves reporting on approximately 200 SSPE patients, with information on date of onset of SSPE, date of measles infection, measles antibody titers and administration of measles vaccine. The analysis has focused on the interval between onset of measles and onset of SSPE and the effect of administration of measles vaccine after measles infection on this interval. Preliminary results have been presented at the meetings of the American Academy of Neurology.

4. Maternal Clinical Infections and Pregnancy Outcome

Principal Investigators: Dr. John L. Sever, Mary Ruth Gilkeson,
and Dr. Jonas H. Ellenberg

Of the approximately 58,000 pregnancies in the Collaborative Perinatal Project, clinical infections during pregnancy were reported in approximately 5000 cases. The children of all pregnancies have been followed in general through the seventh or eighth year of life. The purpose of the study is the analysis of possible associative relationships of infections during pregnancy with pediatric outcomes. The infected mothers have been matched (using race, sex, age, gestation of newborn, sex of newborn, institution and date of last menstrual period) with non-infected controls and comparisons made of rates of specified outcomes. The first phase of the study, which deals with pediatric outcomes through the fourth year of life, has been completed. Preliminary reports have been presented and future publications are expected.

5. Serological Study of Perinatal Infections and Pregnancy Outcomes

Principal Investigators: Dr. John L. Sever, Mary Ruth Gilkeson
and Dr. Jonas H. Ellenberg

This study involves the determination of antibody responses to 11 infectious agents (mumps, rubella, herpesvirus, etc.) using serial sets of maternal sera which have been obtained prospectively from 4000 pregnant women with abnormal pregnancy outcomes and a similar number of matched controls. The general purpose of the study is to obtain information on the effect of maternal, serologically confirmed sub-clinical infections on the fetus and child. The Office of Biometry is actively participating in the planning of the analysis of these data which involves the definition of positive or negative antibody responses to specified antigens, using bloods drawn at varying time intervals and at different times in pregnancy.

6. Primate Information Retrieval System

Principal Investigators: Dr. William T. London, Sebastiano A.
Sciabbarrasi, Kenneth A. Elsner

This system was completed during the year. Its purpose was to establish a computerized file to provide current, research, historical and control information for a large, multiple-site primate colony rapidly and accurately. It also provides a basic information file for the selection of animals and creation of data files for specialized studies of a long-range nature.

Programming was completed by David Smith for the basic system. The principal investigators and their staffs checked and edited the master file for the operating system. Final implementation of the system was completed by the end of the fiscal year.

A paper describing the system was presented at the annual meeting of the American Association for Laboratory Animal Science and was published in Laboratory Animal Science, Vol. 24, No. 5, pp. 773-782.

7. Cebus Monkey Study Data File

Principal Investigators: Dr. A. Palmer, Dr. W. London and Sebastiano
Sciabbarrasi

The purpose of this study is to create a comprehensive research data file of very large magnitude for the efficient conduct of, and for use in analysis of, the long-range Cebus Monkey Research Study in the Infectious Diseases Branch.

This file will base itself on the Primate Information Retrieval System (described above) and will add additional significant variables to that system. A large number of reports have been developed to provide historical, current and analytical reports during the course of the investigation. Programming has been completed and testing and implementation of the system has been started.

8. Evaluation of ^{51}CR Radioimmunoassay

Principal Investigator: Dr. D. L. Madden

Dr. Madden is developing ^{51}CR Radioimmunoassay techniques. His data were analyzed by Mr. Rubinstein to establish the variability of the technique on (a) subsamples of blood drawn from the same sample, (b) samples taken at different times from the same patient, and (c) among patients. As data are accumulated on multiple sclerosis patients and their respective controls, periodic comparisons of their respective immunological behavior are made by various parametric and non-parametric methods.

9. Primate Maternal Nutrition Study

Principal Investigators: Drs. William T. London and Jonas H. Ellenberg

This project involves the study of caloric and/or protein restriction in pregnancy and its effect on biochemistry, and neurological deficits of newborn Rhesus monkeys. The second pilot study has been completed, with 22 out of 24 monkeys successfully impregnated and maintained on their specified diets. Approximately 300 measurements relating to the newborn and the mother have been measured on each monkey. The statistical analysis of possible associations of the studied nutrition factors with outcome of the newborn should be completed by the end of the fiscal year. In addition to the substantive questions to be examined by this study, the study has produced several new results in many previously uninvestigated areas of research in the methodology of primate nutrition.

10. Distribution of Hepatitis B Antigen Serotypes

Principal Investigator: Dr. D. L Madden

Miss Beadle and Mr. Rubinstein reviewed a paper by Dr. David L. Madden, et al, which compared the relative proportions of ad and ay serotypes of Hepatitis B. Antigen among males, females, mongols, and non-mongols. As a result of this review a re-analysis of the data was made to provide comparisons among the groups, separately within wards containing patients of similar ages and intelligence. These separate comparisons were then combined and analyzed to demonstrate possible subgroup differences. Significant differences in ad-ay serotype frequencies were found between males and females and between mongols and non-mongols.

11. Cellular Immunity in Multiple Sclerosis

Principal Investigator: Dr. David A. Fuccillo

It has been reported that patients with multiple sclerosis lack a specific cellular recognition for measles antigen. Dr. Fuccillo has initiated a study of 50 MS patients and their controls in an attempt to confirm this. The lab work has been completed on the subjects and is currently being analyzed in the Office of Biometry.

12. Cytotoxic Antibodies

Principal Investigator: Dr. John L. Sever

Standard computer programs were used to obtain basic statistics on the data provided the Office of Biometry and to determine any relationships between cytotoxic antibodies in mothers and abnormal neurological outcomes in their babies.

Electroencephalography Branch

1. A Study of Simultaneous Monitoring of Changes in Extra-cellular K^+ Concentration and Oxidated Metabolism in Intact Cat Hippocampus

Principal Investigators: Dr. D. Lewis and W. Schuette

Statistical consultation and assistance was provided in determining the relationship of (1) transient fall-off in potassium levels over time in 41 cats; (2) the maximum depression of fluorescence and changes in potassium level; and (3) the area subtended by the fluorescence tangent and changes in potassium level in 6 cats. Data analyses involved determining the proper functions to describe the relationships, regression, regression analysis, analysis of covariance for comparing regression curves, and graphing the curves. A paper, NADH Fluorescence and K^+ Changes During Hippocampal Electrical Stimulation, by Lewis and Schuette was published in Journal of Neurology, 1974.

2. Potassium Kinetics in the Alumina Cream Focus of the Monkey

Principal Investigators: Dr. D. Lewis, Dr. K. Mutsuga, and W.
Schuette

A preliminary study of six monkeys with drug induced seizures was conducted to determine the relationship of the number of fibrous astrocytes to potassium fall-off. The analyses entailed regression analyses, covariance analysis, graphing, etc. There will be more to follow.

3. A Study of Seizure Patterns and Etiopathogenesis in Epileptics

Principal Investigators: Drs. B. Ludwig and C.A-. Marsan

Seizure patterns and etiopathogenesis from EEG examinations of 220 patients with frontal and central origin seizures were compared with those of 55 patients with occipital origin seizures by response (clonic, tonic, automatism, etc.) localization, total groups of responses, and with data from Clinical Ictal Patterns and EEG (Marsan and Goldhammer). These comparisons were made by Chi-square tests of simple and complex contingency tables.

Surgical Neurology Branch

1. Immunochemotherapy in an Intracerebral Murine Glioma Model

Principal Investigators: Drs. J. Madigan and L. Albright

The purpose of this study was to determine the effect of immuno-, chemo-, and immunochemo-therapies on survival and cellular immunity of mice with experimentally induced glioma. Extensive statistical support has been given in evaluation of the adequacy of experimental designs, formulation of the statistical analytical procedure, analysis and interpretation of experimental data, and review of the study report of the first part of this research.

Additional support was provided during the past fiscal year in the determination of sample size and the conduct of statistical analysis of data in a follow-up experiment for an intensive study of the effect of immunotherapy. Data analysis involved evaluation of median survival time, median test, contingency table analysis, analysis of variance, etc. The experimental survival curves for the immunotherapy and control groups were carefully studied and the investigators were advised with regard to the existence of a quality control problem in the experiment.

2. A Study of the Effect of Lobectomy and Craniotomy on Epileptic Patients Electrode Implants

Principal Investigators: Dr. J. Van Buren, Dr. C.A-. Marsan
Dr. N. Mutsuga, and D. Sadowsky

The final analyses and interpretations of these data have been completed, and a report (in which an OB statistician is a co-author) has been published (as a chapter of a book, Advances in Neurology, Vol. 8, 1975) -Surgery of Temporal Lobe Epilepsy by Van Buren, A-. Marsan, Mutsuga, and Sadowsky.

3. A Study of Various Therapies for Malignant Gliomas of the Brain

Principal Investigators: Dr. A. Ommaya, Dr. J. Van Buren, D. Sadowsky

This is a proposed collaborative study including NINCDS, George Washington, and Georgetown Universities. Statistical consultation, assistance, and participation has been and will be given in collecting, coordinating, and analyzing all data from all phases of diagnosis, treatment, and therapy; instruction in and institution of a plan for randomized selection for treatment and therapy; instruction in and setting-up of sequential design and analysis for the trials; conducting data analyses of survival and survival time on rats and later patients; etc.

4. A Study of Arrhythmia in Animals

Principal Investigator: Dr. Mathews

This is a feasibility study of 33 animals that were insulted by a blow to the spinal column. Ten controls received insult only and 23 received other treatments (Stallate, adrenal, vagotomy, and A.V. shunt) in order to determine, by systolic and diastolic blood pressure changes, whether there are differences in both the BP changes and arrhythmia between controls and other treatments, among treatments, between high and low BP's, etc. These differences were determined by t-tests and chi-square tests.

5. A Study of the Behaviors of Epileptic Patients after Temporal Lobectomy

Principal Investigators: Dr. A. K. Ommaya and D. Sadowsky

Statistical consultation and assistance was provided in collecting, analyzing, and interpreting the data (brought up to date from 1964 to the present) for a study of the surgical effect on the behavioral and psychological variables, intelligence, ability to carry on the activities of daily living, and socioeconomic changes of epileptics after surgery. This involves preparing code lists, collecting data, preparation for processing, writing programs, analysis of variance tests, t-tests, regressions, chi-square tests, graphing, etc.

6. A Study of the Effect of Chemical, Surgical, and Radiological Treatment of Pituitary Tumor Patients

Principal Investigators: Dr. A. Ommaya, Dr. C. Strott, and D. Sadowsky

This is a proposed collaborative study, including NICHD and NCI, to investigate which of eight treatments or therapies best normalize the blood values chemically of pituitary tumor patients. Statistical support has been given in the preliminary planning and preparation of the study protocol; instruction in and the setting-up of sequential design and analysis for the clinical trials; and the use of an OB statistician for collector, coordinator, custodian, analyzer, and interpreter of the data.

7. Study of Alteration in the Dentate Nucleus Secondary to Lesions of the Diencephalon

Principal Investigators: M. Levine, D. Sadowsky, Dr. J. Van Buren

This is a continuing project for comparing the results observed from microscopic examination of brain tissue slides of five diencephalic lesioned and seven control patients. Additional data have been collected, analyzed, and interpreted for inclusion in the report in the form of two papers (in which an OB statistician is a co-author) to be sent for publication.

8. A Study of the Long Term Effects of Various Treatment Regimens of Intracranial Gliomas

Principal Investigators: Dr. J. Wood, Dr. R. Rajjout, Dr. A. Ommaya
D. Sadowsky

Statistical consultation and assistance was and will continue to be provided in a study of the long-term effects of treatment of intracranial gliomas in about 100 patients. The statistical support includes formulating the code lists, collecting the data, preparation for processing, and conducting the analyses, interpretations, and reports.

OTHER ORGANIZATIONS

Washington Clinic

1. A Screening Method of Internal Carotid Occlusive Diseases

Principal Investigators: Drs. W. Howell and T.C. Chen

The purpose of this study was to develop a screening method for Internal Carotid Occlusive Diseases with photoelectric plethysmography. Research results obtained from the investigation of 86 subjects were reported previously. A statistical criterion for the screening method was developed. A study report has been drafted for publication. Further study has been carried out to investigate the efficiency of this screening method on bilateral COD patients. Due to the limitation of the research funds of the first investigator, sufficient bilateral COD patients with confirmed carotid angiography have not been collected. The study is to be continued for the next year. This screening method, however, is to be incorporated in a research project of the Bowman Gray School of Medicine Stroke Center.

Mathematical Research Branch, NIAMDD

1. Lumping in Linear Compartments

Principal Investigators: Dr. J.B. Hearon and R.B. Marimont

A study of lumping in linear compartmental systems was begun in collaboration with Dr. John Z. Hearon, Chief, Mathematical Research Branch, NIAMDD. Lumping is the name given to an apparent reduction in the number of compartments in a system; i.e., the reactions in one or more compartments are appropriate to a system of fewer compartments than are actually present.

The on-line modelling laboratory (MLAB) of the PDP10 is suitable for the study, since linear compartmental systems can be solved, the resulting solutions approximated by a smaller compartmental system, and the goodness of fit studied.

It was found, surprisingly, that over a wide range of values, the 2 exponential approximation gave an excellent fit to the 3, 4, and 5 curves, In such cases, the presence or absence of theoretical lumping cannot be determined from the output, particularly in experimental curves which are contaminated with noise. The dependence of goodness of the 2 exponential fit on the parameters of the theoretical curve is being analyzed.

Department of Nutrition, School of Public Health, University of N. Carolina

1. Supplemental Food Program for Women, Infants and Children

Consultants: Mr. Weiss and Miss Jackson

The Office of Biometry provided consultation in the analysis of data from the Medical Evaluation of the Supplemental Food Program of Women, Infants, and Children, conducted by the Department of Nutrition, School of Public Health, University of North Carolina.

This study was designed to evaluate the effects of food supplementation. The consultants, at a series of meetings, examined first preliminary, then final, data.

Duke University

1. Non-Euclidean Graphs in Health Statistics

Principal Investigator: Rosalind B. Marimont

The problem arose in a geriatric health study conducted by Professor Max Woodbury of Duke University. A large sample of patients were rated on 5 factors relating to physical and emotional well-being. A cluster analysis showed a number of typical states and transitions between them, which could be studied by graphs. A property called parallelism was defined for pairs of transitions. It was found that in some cases, the definition led to anomalous results, in that "parallel" edges were incident on the same node. A graph with this property was called non-Euclidean. An algorithm has been devised and programmed to test for the non-Euclidean property, and a general characterization of the property is being sought. It is possible that a redefinition of "parallelism" may be necessary for the best analysis of the data.

RESEARCH

1. Testing for Homogeneity of Variance in a Two-Way Layout

Dr. J. H. Ellenberg of the Office of Biometry is currently doing research on the problem of homogeneity of variance in a two-way layout, where the statistician is faced with determining whether or not the elements of a row or column are sampled from a population with the same variance as the remainder of the rows or columns. To date, the literature dealing with this problem gives tests which are based on large sample approximation theory. An exact test has been developed which can be used for small samples. This work has been submitted for publication.

2. Testing for Outliers in General Linear Regression

Dr. Ellenberg is doing research on the problem of detection of outliers. The problem of detection of outliers in general linear regression was presented in the December 1973 issue of the *Journal of the American Statistical Association.* The focus of the current work is in two areas; first, to show the equivalence of the Ellenberg procedure with other alternatives offered recently in the literature and second, to determine the mechanics of computing the percentage points of the Ellenberg procedure. This work has been submitted for publication.

MISCELLANEOUS ACTIVITIES

1. Editorial Collaboration

Dr. J. H. Ellenberg has regularly served as a reviewer for articles submitted to the statistical journal *Technometrics.*

PUBLICATIONS

Niswander, K., and Jackson, E.C.: Physical characteristics of the gravida and their association with birthweight and perinatal death. *Am. J. Obstet. Gynecol.* 119: 306-313, 1974.

Sciabbarrasi, S. A., and London, W. T.: A computerized primate information retrieval system (PIRS). *Lab. Anim. Sc.* 24: 773-782, 1974.

Sever, J.L., Krebs, H., Ley, A., Barbosa, L.H., and Rubinstein, D.: Diagnosis of subacute panencephalitis, the value and availability of measles antibody determinations. JAMA 228: 604-606, 1974.

Van Buren, J. M., Ajmone-Marsan, C., Mutsuga, N. and Sadowsky, D.: Surgery of temporal lobe epilepsy. Advances in Neurology, Vol. 8, Raven Press, N. Y., 1975.

FUTURE PLANS

1. The Office of Biometry is planning further activities in the area of surveys of neurological diseases and communicative disorders. An RFP for a stroke survey has been advertised. Discussions are underway with the National Center for Health Statistics to include a questionnaire on stroke in their planned Health Interview Survey, which would provide data on questions not otherwise obtainable.

Plans for a survey of epilepsy are expected to be formulated in the next fiscal year.

2. If a contract is let for the planning of a program information system, and its attendant software, the Office of Biometry is likely to be involved as project monitors, and as consultants in the development of the system.

3. With the accession of a neuroepidemiologist to the program, a new program in neuroepidemiology will be initiated.

4. The Office of Biometry, in collaboration with investigators in the Intramural Program, plans to develop a proposal for the development of a Pain Data Center in NINCDS, which will also provide a source for clinical research, and for statistical mathematical investigations concerning the quantitative expression of pain.

5. The Office of Biometry is considering the acquisition of a mini-computer and software to permit the interactive calculation and solution of statistical problems.

Advantages of this system are:

1. ready accessibility to statisticians.
2. simplified programming languages (BASIC).
3. costs which will be lower than present expenditures.

OW TO USE
HESE SEPARATORS

se one page for
ach separation.

elect appropriate
ab, add further
lentification if
sired, and cover
t with scotch
pe.

it off and discard
l tabs except the
ie covered by tape.

ABBED SEPARATOR SHEET
orm HEW-69
3-56)

ANNUAL REPORT
For Period July 1, 1974 through June 30, 1975
Perinatal Research Branch
Collaborative and Field Research
National Institute of Neurological and Communicative Disorders and Stroke
National Institutes of Health

TABLE OF CONTENTS

Page No.

I. General Summary.. 1

II. Contract Narratives

University of Wisconsin (N01-NS-2-2302)
Genetic-odontometric Study of Pre- and Neonatal Growth.. 9

University of Michigan (N01-NS-2-2320)
Bivariant Physical Growth.. 11

Johns Hopkins University (N01-NS-2-2321)
Ancillary Studies.. 13

Boston University (N01-NS-2-2322)
Maternal Drug Ingestion and Fetal Abnormalities............ 15

Pennsylvania State University, M.S. Hershey Medical Center (N01-NS-3-2311)
Analysis of General Pathology and Placental Pathology of the Collaborative Perinatal Project Data.. 17

Children's Hospital Medical Center, Boston, Massachusetts (N01-NS-3-2312)
Combined Neuropathologic and Epidemiologic Study.......... 19

Beth Israel Hospital, Boston, Massachusetts N01-NS-3-2320)
A Dynamic Time Trend Analysis of Collaborative Perinatal Project Data on Patterns of Blood Pressure, Edema and Proteinuria (Toxemia) as Related to Pregnancy Outcomes.............................. 21

University of Minnesota (N01-NS-4-2326)
Analysis of Speech, Language and Hearing Deficits to Facilitate Prevention, Diagnosis and Treatment.......... 23

TABLE OF CONTENTS (cont'd)

Page No.

III. Individual Project Reports.................................... 25

IV. A Comprehensive Plan for Analysis and Interpretation of Collaborative Perinatal Project Data

Primary Data Analysis Areas

Cerebral Palsy.................................... 63
Mental Retardation.................................... 69
Communicative Disorders.................................... 23
Visual Abnormality.................................... 73
Convulsive Disorders.................................... 61
Learning Disorders.................................... 75
Minimal Brain Dysfunction.................................... 67
Congenital Malformations.................................... 79
Birthweight-Gestational Age Relationships (Prematurity).................................... 65
Neuropathology, General Pathology and Placentology.................................... 17, 19

Secondary Data Analysis Areas

Toxemia.................................... 21
Maternal Infection During Pregnancy (See report by Infectious Diseases Branch, C&FR, NINCDS)
Neonatal Hyperbilirubinemia.................................... 87
Maternal Anesthesia-Analgesia During Labor and Delivery.................................... 89
Four-Year IQ.................................... 51
Physical Growth and Development (Birth to Seven Years).................................... 11
Twins (To be developed)
Genetic and Socio-economic Factors.................................... 35,43,47
Drugs Taken During Pregnancy.................................... 15
Labor and Delivery (To be developed)

ANNUAL REPORT
For Period July 1, 1974 through June 30, 1975
Perinatal Research Branch
Collaborative and Field Research
National Institute of Neurological and Communicative
Disorders and Stroke
National Institutes of Health

GENERAL SUMMARY

1. INTRODUCTION:

The Collaborative Perinatal Project (CPP) is a longitudinal multidisciplinary research effort which seeks leads to the etiologies of cerebral palsy, mental retardation, learning disorders, congenital malformations, minimal brain dysfunction, convulsive disorders and communicative disorders through studies which relate the events, conditions, and abnormalities of pregnancy, labor and delivery to the neurological and mental status of the children of these pregnancies as the child grows and develops. Data collection has been completed, and the major emphasis of the project has shifted to data analysis and interpretation for publication of reports on the CPP research findings. In order to accomplish this, a coordinated effort has been implemented to meet the basic data analysis objectives of the CPP. To provide a framework for this effort, a Comprehensive Plan for Analysis and Interpretation of Collaborative Perinatal Project Data has been approved and implemented.

I. DATA COLLECTION:

The Collaborative Perinatal Project was initiated in 1959 when the women began registering at each of the collaborating institutions during their pregnancies. Women continued to enroll in the study through December of 1965. The babies were delivered between 1959 and 1966 and received several examinations during their first year of life, at 3 years of age, and at 4 years of age. The 7-year extensive battery of examinations included a pediatric-neurological examination; a battery of psychological tests, including an IQ determination; and a visual screening test. By June of 1974, an assessment of speech, language and hearing development was completed on children eight years of age at five of the collaborating institutions. Completion of the eight-year examination terminated follow-up of CPP children. During the current fiscal year, the last of these examination protocols has been edited, coded and the data punched onto computer tape completing the data file of the CPP.

I. A COMPREHENSIVE PLAN FOR ANALYSIS AND INTERPRETATION OF COLLABORATIVE PERINATAL PROJECT DATA:

Broadly stated, the Collaborative Perinatal Project is concerned with the identification of prenatal factors that have sufficiently high association with adverse pregnancy outcome and subsequent neurological and mental development of the child to provide leads to the etiologies of the abnormalities and thus to the development of strategies for prevention and intervention. After a careful review of the objectives of the Collaborative Perinatal Project, the data available for analysis and the work in progress, it was recommended that major efforts in analysis and interpretation were needed in ten primary areas in order to meet the basic objectives of the project. Monograph reports in book form are planned in each of the following primary areas:

Cerebral Palsy (See Individual Project Report - Project No. Z01 NS 02059-03 PR)

Mental Retardation (See Individual Project Report - Project No. Z01 NS 02106-02 PR)

Communicative Disorders (See Contract Narrative N01-NS-4-2326)

Visual Abnormality (See Individual Project Report - Project No. Z01 NS 02107-02 PR)

Convulsive Disorders (See Individual Project Report - Project No. Z01 NS 02058-03 PR)

Learning Disorders (See Individual Project Report - Project No. Z01 NS 02108-02 PR)

Minimal Brain Dysfunction (See Individual Project Report - Project No. Z01 NS 02062-03 PR)

Congenital Malformations (See Individual Project Report - Project No. Z01 NS 02109-02 PR)

Birthweight-Gestational Age Relationships (Prematurity) (See Individual Project No. Z01 NS 02060-03 PR)

Neuropathology, General Pathology and Placentology (See Contract Narratives, Contracts N01-NS-3-2311 & N01-NS-3-2312

IV. IMPLEMENTATION OF THE COMPREHENSIVE PLAN:

Implementation of the Comprehensive Plan for Analysis and Interpretation of Collaborative Perinatal Project Data is carried out through teams of researchers, headed in each of the ten primary areas by a member of the Professional Staff Consultants Section of the Perinatal Research Branch. On each team, there is a member of the Office of Biometry staff, who participates fully in the development of the analysis. In addition, two members of the Section for Production of Data Analysis, Perinatal Research Branch, are assigned to each primary area to facilitate data processing. The assignments are as follows:

RIMARY DATA ANALYSIS AREAS	BRANCH	OFFICE OF BIOMETRY
Cerebral Palsy	Dr. K. B. Nelson	Dr. J. H. Ellenberg
Mental Retardation	Dr. S. H. Broman	Dr. P. W. Shaughnessy*
Communicative Disorders	Dr. P. J. Labenz	Mr. D. Rubinstein
Visual Abnormality	Dr. R. Feinberg	Miss E. C. Jackson
Convulsive Disorders	Dr. K. B. Nelson	Dr. J. H. Ellenberg
Learning Disorders	Dr. S. H. Broman	Dr. P. W. Shaughnessy*
Minimal Brain Dysfunction	Dr. P. L. Nichols	Dr. T. C. Chen
Congenital Malformations	Dr. N.C. Myrianthopoulos	Mr. D. Rubinstein
Birthweight-Gestational Age Relationships (Prematurity)	Dr. Bill H. Williams	Miss E. C. Jackson
Neuropathology, General Pathology and Placentology	Dr. J. S. Drage	Dr. T. C. Chen

In each of the ten primary areas, a program plan has been developed and approved which expands in detail on the summary statements in the Comprehensive Plan and gives a detailed approach to the analysis and identifies major components. Written monthly reports are prepared and meetings held to record progress and identify problem areas. In order to facilitate the primary data analyses and/or complete major efforts well underway, additional analyses are to be completed in the following secondary areas:

Toxemia (See Contract Narrative, Contract N01-NS-3-2320)

Maternal Infection during pregnancy (See report by Infectious Diseases Branch, C&FR, NINCDS)

Neonatal Hyperbilirubinemia (See Individual Project Report - Project No. Z01 NS 02112-02 PR)

Maternal anesthesia-analgesia during labor and delivery (See individual project report - Project No. Z01 NS 02169-01 PR)

No longer with the Office of Biometry, but continues to serve as a Consultant to this area.

Four-Year IQ (See Individual Project Report -
Project No. Z01 NS 01968-04 PR

Physical Growth and Development (Birth to Seven Years) (See Contract
Narrative, Contract N01-NS-2-2320

Twins (To be developed)

Genetic and Socio-economic Factors (See Individual Project Reports -
Project Nos. Z01 NS 01514-09 PR, Z01 NS 01857-06,
Z01 NS 01910-05 PR)

Drugs taken during pregnancy (See Contract Narrative, Contract N01-NS-2-2322)

Labor and Delivery (To be developed)

V. SUMMARY OF WORK IN PROGRESS:

In the cerebral palsy area, children with cerebral palsy have been identified and classified. Files are nearing completion which will permit the computerized screening of antecedent maternal-obstetrical variables and pediatric variables with regard to their association with these cerebral palsy diagnoses. The double disability of cerebral palsy and severe mental retardation is being investigated. Ninety children so disabled have been identified. Four groups of neonatal factors appear especially important: (1) small size at birth, (2) difficulty in independent respiration, (3) low hemoglobin and hematocrit, and (4) neonatal seizures. A manuscript has been completed which relates neurological findings at one year with subsequent cerebral palsy diagnosis at age seven: children with mild, early CP often lose their motor deficits by school age, but remain at heightened risk for intellectual subnormality. Useful predictors of status at school age have been identified for this group.

In the mental retardation area, preliminary findings indicate that the following conditions relate to a low level of intellectual functioning at age seven: low socioeconomic status, high housing density, low maternal intelligence, less parental care, more neonatal complications, less advanced mental and motor development at 8 months, more frequent neurological signs at one year, poorer language development at age three, lower psychometric test performance at age four, and smaller physical size during the first 7 years of life.

Studies in the area of communicative disorders are being conducted under a single contract. The associations between speech, language and hearing (SLH) findings and other CPP variables are being analyzed. The aim of these studies is to provide clues to the etiology of communicative disorders, and toward clinical applications and prediction. An evaluation of the quality of the SLH data is nearing completion. Key SLH variables will then be identified and composite indices constructed to serve as summary descriptors of communicative status.

In the area of visual abnormality, basic tabulations of selected outcome variables have been received and are being analyzed. Case histories of unusual visual abnormalities will receive anecdotal treatment and are currently being hand-reviewed.

In the convulsive disorders area, case review has been completed on approximately 2,700 records of children thought to have experienced convulsive disorders. This clinical material has been classified and coded and is being prepared for tape file creation, thus readying the data for computer analysis.

Preliminary findings in the learning disorders area indicate that children with learning disorders came from families of lower socio-economic status; the mothers were of higher parity, received less prenatal care, and were more frequently unmarried. Speech was less well developed at age three and psychological test scores lower at age four. Hyperactivity, shorter attention span, and less verbal communication were evident at age four. Similar findings emerged at age 7 even when IQ was matched in the controls.

In the area of minimal brain dysfunction, correlations and factor analyses of individual symptoms have identified four major factors that will be studied in detail. These include academic problems, minor neurological problems, hyperactivity-impulsivity, and "socio-emotional immaturity".

The congenital malformations area is divided into 11 segments, three of which have been published or are in press. The remaining segments are in progress or in the design stage. Selected findings in the overall analysis are as follows: Only a third of malformations observed during the first year of life were diagnosed at birth. Twins have more malformations than singletons but the difference is wholly contributed by monozygotic twins; monoamniotic twins have more malformations than diamniotic. Multiple birth, pregnancy complications (mostly hydramnios) and male birth were associated with an increased risk in major malformations. Children of chronic alcoholic mothers show a constellation of developmental defects known as the "fetal alcoholic syndrome". Strong associations have been detected between blood groups and malformations. Multiple malformations are being reviewed; a preliminary analysis of the first 212 cases showed that 14% represent known syndromes, 54% are primary single malformations, and 32% remain unclassified.

In the birthweight-gestational age area the use of polynomial regression analysis has examined the relationships of birthweight to secondary parameters including placental weight, maternal education, socioeconomic index, gestation at registration, year of birth, institution, body length and head circumference at birth. A covariance program has been developed to study the relationship of birthweight to placental weight and to gestational age, for survivors and non-survivors. A multivariate analysis will be pursued to determine the relationship of these variables to antecedent factors and later neurological outcome.

Studies in the area of pathology are being undertaken by two separate contracts, one for neuropathology and one for the general and placental pathology. The neuropathology contractor will prepare a catalogue of gross brain abnormalities, plot fetal brain weight against gestational age, and analyze the events of pregnancy, labor and delivery for those brains deviating from the projected growth curve. Intra-and extra-utero growth rates will be compared. Intra-cranial hemorrhage, telencephalic leucoencephalopathy, and cerebral necrosis will be studied in detail and related to possible risk factors. The general and placental pathology contractor will review deaths and classify associated factors. Demographic characteristics, maternal conditions and events of pregnancy, labor and delivery will be related to deaths. Organ growth rates will be analyzed and related to maternal conditions as contributory factors. Placental abnormalities will be classified and related to demographic characteristics, maternal conditions, and events of pregnancy, labor and delivery. Placental weight and placental pathology will be analyzed with respect to birthweight-gestational age classifications and pregnancy outcome. In the neuropathology area approximately 20,000 slides have been reviewed as the preparation, review and coding of the histological material for analysis continued in Fiscal Year 1975. In the general pathology area 39,780 cases have been reviewed to confirm or reclassify diagnoses of placental slides.

The study of "Toxemia of Pregnancy" continued in Fiscal Year 1975 under contract. The objective is to establish a precisely defined classification by a time-trend analysis of the data pertinent to clinical diagnosis: blood pressure, proteinuria, and edema. The first two phases of the analysis which delineate separately the patterns of blood pressure, proteinuria, and edema have been completed. Adverse pregnancy outcome was associated with rapid changes or high levels of both blood pressure and proteinuria. Phase III is well under way and will result in a synthesis of the first two phases.

The neonatal hyperbilirubinemia study was implemented during Fiscal Year 1974. This study has been designed to assess the relationships of intermediate (10-20 mg.%) levels of serum bilirubin on subsequent neurological and mental development of CPP children. The analysis of phase I has been completed, and a manuscript is in preparation.

A study of the relationship between intelligence at four years of age, as measured by the Stanford-Binet Intelligence Scale, and a variety of factors related to pregnancy, delivery, postnatal status, and environment, was published during Fiscal Year 1975. Broman, S.H., Nichols, P.L., and Kennedy, W.A.: Preschool IQ: Prenatal and Early Developmental Correlates. Hillsdale N.J.: Lawrence Erlbaum Associates (distributor, Halsted Press, Jonn Wiley & Sons, New York) 1975, 360 pp.

Detailed analysis of physical growth data on CPP children to seven years of age continued in Fiscal Year 1975 under contract. Under consideration are weight, height, head circumference, and chest circumference. Analyses are dimensional, incremental, and bivariate and the analysis will be by race, sex, and socioeconomic groupings. The need for a more comprehensive analysis has been demonstrated and an expanded contract is being negotiated.

Additional work in the Family Linkage file resulted in a refined and more precise file. This file is nearing completion for use in other program areas.

The study of the effects of maternal drug ingestion on perinatal deaths and congenital malformations continued during Fiscal Year 1975 under contract. The study will describe drug utilization patterns during pregnancy and provide for a rapid evaluation of drug teratogenicity through the use of a screening device to calculate relative risk. Other relationships are being explored and a number of papers have been published. The drug exposure file has been completed. Several dozen associations between drugs and malformations have emerged from multiple logistic analysis, as well as from the specially standardized drug exposure screens. These leads are being pursued.

I. CONTRACT DEVELOPMENT:

During this Fiscal Year a contract was developed and awarded for the microfilming of the PRB records. It is projected that by the end of the year approximately 15,000 records will have been processed for filming. A microfilm system for retrieval of information by researchers is planned to replace the present manual system for retrieval of original records.

A contract is being developed in the physical growth area, one of the secondary areas within the Comprehensive Plan. The analyses to date strongly indicated that a more comprehensive approach to the data is required. The results of this analysis will serve as valuable input to other study areas.

The correlation of minor chromosomal variants with congenital malformation is also being developed as a contract project. This project will utilize data collected by five of the Collaborative Perinatal Project Institutions, which are participating in a chromosomal study of minor variants. The collection of the chromosomal material was sponsored by the National Institute of Child Health and Human Development.

II. SUPPORT FUNCTIONS:

The Section for Data Collection is responsible for processing research protocols. During the first quarter of this fiscal year the last study forms were received. All study records received for processing have been edited, coded and key punched. Work continues on 7-year write-in data, filing the backlog of forms, and correcting the Master Data File.

The Section for Production of Data Analysis continues to perform automatic data processing: updating of the Master Data File, creating special work tapes for the various studies and processing data requests from researchers. During the year the Master Data File underwent resequencing and modifying in order to provide for more efficient updating. A special statistical analysis of covariance system is under development.

CONTRACT NARRATIVE
Perinatal Research Branch, C&FR, NINCDS
Office of the Chief
July 1, 1974 through June 30, 1975

UNIVERSITY OF WISCONSIN: (N01-NS-2-2302)

Title: Genetic-odontometric study of pre and neonatal growth

Contractor's Project Director: Richard Osborne, Ph.D.

Current Annual Level: $26,476.00

Objectives: To correlate the incidence and prevalence of dental and facial abnormalities with neurological defects, congenital abnormalities and other disorders of childhood.

Methodology: Dental casts and photographs of teeth will be obtained on about 2500 Collaborative Project children who are scheduled for routine seven and eight year examinations at selected participating institutions, and on children rescheduled because of known complications during their prenatal and neonatal periods of study.

Current Status: Collection of casts and film on 2217 children has been completed. All dental measurements have been taken and discrepancies have been resolved. All observations and measurements have been put on tape. It appears that over 20 percent of children in this sample have dental abnormalities. A preliminary analysis has been made to partition out the genetic variance in order to exclude it from the final analysis, which is in progress.

Significance to the Program: The study of dental abnormalities is expected to yield an index of stress during prenatal development.

Proposed Course of the Contract: The contract expires May 31, 1975. No further extension of the contract is anticipated.

CONTRACT NARRATIVE
Perinatal Research Branch, C&FR, NINDS
Office of the Chief
July 1, 1974 through June 30, 1975

UNIVERSITY OF MICHIGAN (NO1-NS-2-2320)

Previous Contract Number: NIH-NINDS-72-2320

Title: Bivariant Physical Growth

Contractor's Project Director: Stanley M. Garn, Ph.D.

Current Annual Level: $16,900

Objectives:

a. Merge per-capita income and socio-economic index data per individual with anthropometric data on tapes and; after checking, convert to MIDAS for the most economical approach to b. through e. below.

b. For 4 anthropometric variables, (A, weight; B, height; C, head circumference; D, chest circumference) by 2 sexes, by 2 races by 3-4 economic groupings, compute new percentiles for at least 2 ages to see how the percentiles and therefore the medians are affected, within a race and between races at comparable economic groupings and between economic groupings.

c. For 4 anthropometric variables and a single later age, for 2 sexes, by 2 or more races, compute new mini bivariant tables. A by B, A by C, A by D, B by C, B by D, C by D, for each of 3-4 economic groupings and thus ascertain the extent to which income effects size percentiles A by size intervals B by constant age.

d. For a set of ages, including the ages used in b. and c. compute the increments (deltas) for the same 4 dimensional variables given in b. and c. and percentiles for the deltas, by sex, race, and 3-4 socio-economic groupings to ascertain: the effect of income, income/needs or socio-economic index on deltas, within races and for comparable groupings, between races.

e. Using a special MIDAS program, convert the low-income skewed sample into a more nearly national probability sample, by weighing, so that one set of "norms" can be developed that will not be simply limited to the original lower-income weighted distributions. Specifically, percentiles (see b. above) and increments (see d. above).

Major Findings: Detailed analysis of PRB physical growth data (dimensional, incremental, and bivariant) show the need for a) excluding improbable, miscoded, or mispunched values, b) meaningful arrangement by PRB SE Index and other measures of social class, c) corrected and uncorrected birth weight groupings, and d) PRB SE Index in developing bivariant tables. That is, achievement of the above objectives indicates the need for a more comprehensive analysis of the physical growth data.

This contract has been terminated. A new contract is being sought.

Course of Contract: September 28, 1973 through September 27, 1974.

CONTRACT NARRATIVE
Perinatal Research Branch, C&FR, NINCDS
Office of the Chief
July 1, 1974 through June 30, 1975

JOHNS HOPKINS UNIVERSITY (N01-NS-2-2321)

Title: Ancillary Studies

Contractor's Project Director: Janet B. Hardy, M.D.

Current Annual Level: $51,600

Objectives: Using NINCDS Collaborative Perinatal Project data and data unique to the Johns Hopkins Collaborative Perinatal Study, the contractor will conduct studies in the following areas:

a. Perinatal infections.
b. Problems in communication.
c. Growth and development of the child in an inner-city population.
d. The psychological, biological and environmental factors relating to IQ.
e. The etiology and characterization of seizures.
f. The effect of maternal smoking on the fetus.
g. The toxicity of low levels of bilirubin.

The contract has been ammended to include the following:

1.) Full participation by the Project Director in the analysis and interpretation of the data output from the birthweight-gestational age study to result in a manuscript suitable for publication as a monograph.

2.) Full participation by the Project Director in the analysis and interpretation of the data output of Phase I from the hyperbilirubinemia study.

Major Findings: See the following individual project reports.

1.)	Birthweight-gestational age.	Project No. N01 NS 02060-3 PR
2.)	Hyperbilirubinemia.	Project No. N01 NS 02112-02 PR
3.)	The First Year of Life.	Project No. N01 NS 02052-03 PR

The study of young fire setters has been completed. Of 15 children among approximately 3,000 surveyed who had set major fires by age 8 years, 67 percent had mothers below 17 years of age, there was a significant increase in signs suggesting subtle brain damage, and school performance was poor in relation to IQ scores obtained.

Course of Contract: June 28, 1972 through June 27, 1975.

Publications: Hardy, J. B.: Birth weight and subsequent physical and intellectual development. N. Engl. J. Med. 289:973-974, 1973.

Hardy, J. B. and Mellits, E. D.: Does maternal smoking during pregnancy have long term effect on the child? Lancet. 2:1332-1336, 1972.

Hardy, J. B.: Clinical and developmental aspects of congenital rubella. Arch. Otolaryngol. 98:230-236, 1973.

Wolff, S. M.: The ocular manifestations of congenital rubella. A prospective study of 328 cases of congenital rubella. J. Pediatr. Ophthalmol. 10:101-141, 1973.

Welcher, D. W., Wessel, K. W., Mellits, E. D. and Hardy, J. B.: The Bender-Gestalt test as an indicator of neurological impairment in young "inner city" children. Percept Mot. Skills. 38:899-910, 1974.

Hardy, J. B.: Congenital rubella. In Ryan, S. J., Jr. (Ed.): Eye and Systemic Disease. New York, Grune & Stratton, 195-204, 1974.

Hardy, J. B.: Rubella and Congenital Rubella. Current Therapy. W. B. Saunders Company, 57-60, 1974.

CONTRACT NARRATIVE
Perinatal Research Branch, C&FR, NINCDS
Office of the Chief
July 1, 1974 through June 30, 1975

BOSTON UNIVERSITY (N01-NS-2-2322)

Title: Maternal Drug Ingestion and Fetal Abnormalities

Contractor's Project Director: Dennis Slone, M.D.

Current Annual Level: $165,000

Objectives: Using NINCDS Collaborative Project data, the objective of the contract is to conduct a research program on maternal drug ingestion and fetal abnormalities. The study will describe maternal drug utilization patterns, provide for a rapid evaluation of drug teratogenicity, report on the relationship between Dilantin and selected congenital malformations, investigate the possible teratogenic effects of virus vaccines, and investigate possible associations between drugs and congenital cardiac malformations.

Major Findings: No evidence was found for a relationship between antenatel exposure to meprobamate and chlordiazepoxide and an increased incidence of malformations in the Perinatal Project population. A preliminary screening analysis between antenatal drug exposure and an outcome of cerebral palsy was completed and several associations emerged, particularly involving the opiate group of drugs. The drug exposure file has been completed. Several dozen associations between drugs and malformations have emerged from the multiple logistic analyses, as well as from the specially standardized drug exposure screens. These leads will be pursued during the last three months of the contract.

Course of Contract: June 28, 1972 through June 27, 1975

Publications: Heinonen, O. P., Shapiro, S., Monson, R. R., Hartz, S. C., Rosenberg, L. and Slone, D.: Immunization during pregnancy against poliomyelitis and influenza in relation to childhood malignancy. Int. J. Epidemiol. 2:229-235, 1973.

Monson, R. R., Rosenberg, L, Hartz, S. C., Shapiro, S., Heinonen, O. P. and Slone, D.: Diphenylhydantoin and selected congenital malformations. N. Engl. J. Med. 289:1049-1052, 1973.

Slone, D., Heinonen, O. P., Monson, R. R., Shapiro, S., Hartz, S. C. and Rosenberg, L.: Maternal drug exposure and fetal abnormalities. Clin. Pharmacol. Ther. 14:648-653, 1973.

The Boston Collaborative Drug Surveillance Program (Manuscript prepared by Olli P. Heinonen): Diethylstilbestrol in pregnancy. Frequency of exposure and usage patterns. Cancer. 31:573-577, 1973.

Hartz, S. C., Heinonen, O. P., Shapiro, S., Siskind, V. and Slone, D.: Antenatal exposure to meprobamate and chlordiazepoxide in relation to malformations, mental development, and child mortality. The New England Journal of Medicine. 292: 726-728, 1975.

CONTRACT NARRATIVE
Perinatal Research Branch, C&FR, NINCDS
Office of the Chief
July 1, 1974 through June 30, 1975

PENNSYLVANIA STATE UNIVERSITY, M.S. HERSHEY MEDICAL CENTER (N01-NS-3-2311)

Title: Analysis of General Pathology and Placental Pathology of the Collaborative Perinatal Project Data.

Contractor's Project Director: Richard L. Naeye, M.D.

Current Annual Level: $136,500

Objectives: This contract is in two parts. One part concerns the general pathology and the other part the placental pathology.

The Objective of the general pathology analysis is to review and classify deaths as to the cause of death, factors contributing to death, and other associated factors. The pathologist is making an assessment of morphologic diagnoses using Collaborative Perinatal Project (CPP) protocols, magnetic data tapes and histologic tissues and slides. An analysis will be completed in the following areas: 1) demographic characteristics (age, race, marital status and others deemed pertinent to a pathology-oriented analysis) of women to develop possible associations with stillbirth, neonatal, and later death; and other classifications of specific pathological or anatomical findings deemed relevant to demographic characteristics. 2) maternal conditions and events of pregnancy, labor and delivery to test for associations with stillbirth, neonatal, and later deaths, and with specific pathological and anatomical findings or groups of findings thought by the pathologist to need exploration. For neonatal and later deaths, data obtained on the child prior to death will be examined. Such analysis will include specific conditions; such as, hyaline membrane disease, erythroblastosis, congenital malformations, and sudden unexplainable death. 3) organ growth rates, including the quantitative extent to which a variety of reported maternal conditions and events may modify or contribute to altered organ growth rates.

The Objective of the placental pathology analysis is to review and classify the various placental findings as reported on study protocols (Path-1 and Path-2), by using the protocols, magnetic data tapes, and placental microscopic slides and tissues. Through such a review, the pathologist will be expected to define and create additional classifications of placental pathology. Analyses will be completed on 1) demographic characteristics and maternal conditions and events of pregnancy, labor, and delivery by the classical and innovative pathological and anatomic categories established by the pathologist; and 2) birthweight-gestation classifications by placental weight, placental pathology and pregnancy outcome.

Major findings: A primary review of placental slides to confirm or reclassify diagnoses on 39,780 cases in the study has been completed. Sudden Infant

Death Syndrome (SIDS) cases have a larger than anticipated number of abnormalities upon post mortem examination. A multivariate analysis has been completed on 28 major maternal and fetal factors that contributed to fetal and infant deaths.

Course of Contract: June 1, 1973 through June 30, 1976.

CONTRACT NARRATIVE
Perinatal Research Branch, C&FR, NINCDS
Office of the Chief
July 1, 1974 through June 30, 1975

CHILDREN'S HOSPITAL MEDICAL CENTER, BOSTON, MASSACHUSETTS (N01-NS-[illegible])

Title: Combined Neuropathologic and Epidemiologic Study

Contractor's Project Director: Floyd H. Gilles, M.D.

Current Annual Level: $206,150

Objectives: The contract will analyze the neuropathology collection of the Collaborative Project (CPP). A safe, readily accessible site for the neuropathology collection will be created to receive the material formerly housed in the Perinatal Research Branch, C&FR, NINCDS, Bethesda, Maryland. An estimate of the quality of the material and a catalogue of gross brain abnormalities will be prepared. Plots of fetal brain weight of grossly normal brains against estimated gestational age, utilizing a Gompertz funtion, will be made. For brains lying beyond one and/or two standard deviations from the growth curve projected by the model, an analysis will be made of events of pregnancy, labor, and delivery. A comparison will be made of rate of brain weight acquisition in utero to rate of brain weight acquisition after birth as a function of total (gestational plus survival) age. A study will be made of intracranial hemorrhage including topography of hemorrhage (ventricular, within plexus; ventricular, external to plexus; ganglionic eminence' extraventricular within brain; arachnoidal; extra-arachnoidal), time of age at death, incidence of venus thrombi, incidence of Galenic or sinus thrombi, incidence of venous dilation, and evaluation of such risk factors as pulmonary disease, cardiac diseases, liver and spleen weight, fetal experiences, and body and brain weight. A study will be done on the risk factors associated with perinatal telencephalic leucoencephalopathy to test the following hypotheses: 1) infants with perinatal telencephalic leucoencephalopathy differ from those without it in regard to (a) immunoglobins and (b) maternal immunoglobins; and 2) infants with perinatal telencephalic leucoencephalopathy differ from those without in regard to thymus weight and morphology. A study of cerebral necrosis is to be completed which would include criteria of necrosis in the perinatal brain, neuronal necrosis (relative maturity of neuronal aggregate; relative immaturity of neuronal aggregate, topography; modes of repair), white matter necrosis (necrotic foci; topography; modes of repair) and an evaluation of selected risk factors in relation to subclassification of neuronal and white matter necrosis.

Major Findings: Preparation, review, and coding of the histological material for analysis continues. Approximately 20,000 slides have been reviewed. A Gompertz function has been found to fit the Perinatal Project material well when fetal brain weight is plotted against gestational age, with correlation coefficients ranging from 0.77 to 0.88. This compared favorably to that obtained from a Children's Hospital Medical Center model.

Course of Contract: June 1, 1973 to June 30, 1976.

CONTRACT NARRATIVE
Perinatal Research Branch, C&FR, NINCDS
Office of the Chief
July 1, 1974 through June 30, 1975

BETH ISRAEL HOSPITAL, BOSTON, MASSACHUSETTS (N01-NS-3-2320)

Title: A Dynamic Time Trend Analysis of Collaborative Perinatal Project Data on Patterns of Blood Pressure, Edema and Proteinuria (Toxemia) as Related to Pregnancy Outcomes.

Contractor's Project Director: Emanuel A. Friedman, M.D.

Current Annual Level: $165,000

Objectives:

The objective of the "Toxemia of Pregnancy" contract is to establish a precisely-defined toxemia classification by an analysis and interpretation of Collaborative Perinatal Project (CPP) data pertinent to the clinical diagnosis of toxemia: blood pressure, proteinuria and edema. The objective of the CPP is to provide leads to the etiologies of neurological and mental abnormalities of children through a study of the conditions and events of pregnancy, labor and delivery. Toxemia is one major abnormality of pregnancy, which is known to have an increased risk to the fetus of death or premature birth with attendant risk to the survivors. However, toxemia has been vaguely and inconsistently defined and this research effort is intended to classify the varying degrees of toxemia so that each precisely-defined degree of toxemia can be assessed for a possible etiological relationship with neurological and mental abnormality of the child.

An estimated 5% to 10% of CPP women have manifested one or more symptoms of toxemia. Extensive data on systolic and diastolic pressure, varying degrees of proteinuria and degrees of edema have been collected on CPP women.

The research as detailed in the work scope plan contains a dynamic time-related trend analysis of blood pressure, edema, and proteinuria; and intercorrelations of the preceding factors. The dynamic time-related trend analysis for each of the factors would include the determination of patterns over the course of pregnancy for individual gravidas, categorizing the trend patterns, establishing relationships to maternal and pregnancy outcome factors, and defining the relative risk effect for each trend pattern. Another major analysis would be a multiple regression analysis of contributory factors for each of the toxemia classifications.

Major Findings: Phases I and II of the planned analyses are complete and their synthesis is well underway in Phase III. The elements of the current analysis are those patterns found during the earlier two phases and involve the following "Toxemia Variables": birthweight, systolic blood pressure, diastolic blood pressure, proteinuria scale, and edema scale. During phases I and II associations were found between adverse pregnancy outcome and large maternal weight losses (more than 1lb/wk) but not excessive weight gain.

Very high and/or rising blood pressures and very low and/or falling pressures affect outcome adversely. Similarly, proteinuria of 2 + or more was consistently associated with adverse fetal outcome, and to a lesser extent with a lower 4 year IQ.

Course of Contract: June 25, 1973 through January 31, 1976.

CONTRACT NARRATIVE
Perinatal Research Branch, C&FR, NINCDS
Office of the Chief
July 1, 1974 through June 30, 1975

UNIVERSITY OF MINNESOTA (N01-NS-4-2326)

Title: Analysis of Speech, Language and Hearing Deficits to Facilitate Prevention, Diagnosis and Treatment

Contractor's Project Directors: Frank M. Lassman, Ph.D., and
Robert O. Fisch, M.D.

Current Annual Level: $219,932

Objectives: Data collected in the Collaborative Perinatal Project (CPP) will be analyzed for associations existing among speech, language and hearing (SLH) findings and variables in other areas of study. These include variables relating to pregnancy, labor and delivery, family characteristics, and the physical, mental and behavioral characteristics of the children. The associations among variables will be studied with an aim toward providing clues to the etiology of communicative disorders, and toward clinical applications and prediction as well.

In order to facilitate study of the relationships with CPP variables, key SLH variables will be identified and composite indices constructed to serve as summary descriptors. Evaluation of the quality of SLH data will precede the selection of these indicators of communicative competence and status. Certain relationships of particular interest will be the subjects of supplemental analyses. Some examples of these are the relationships between respiratory and middle ear infections to hearing level, between speech intelligibility and phoneme articulation, motor development and speech development, and the like.

Results of the analyses and the interpretation of their significance and applicability will be made available by publication as a monograph.

Course of Contract: June 29, 1974 through June 28, 1976.

Project No. Z01-NS-01163-13-PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: An Investigation into the Relationship Between Congenital Heart and Great Vessel Anomalies and Selected Factors as Recorded in the Collaborative Perinatal Research Project.

Previous Project Number: NDS (CF)-63 PR/PSC 1163

Principal Investigator: Lenore Bajda, M.D., PRB, NINCDS

Other Investigators: None

Cooperating Units: Office of the Director, NHLI

Man Years:

Total:	.20
Professional:	.20
Other:	.00

Project Description: The primary objective of this study is to assess relationships between certain maternal variables and congenital heart-great vessel anomalies.

Additional objectives include investigating relationships between early signs of abnormality and the existence of definitive congenital heart lesions and determining the existence of congenital heart-vessel anomaly in conjunction with mental retardation as recorded in the eight-month psychological examination, the one-year summary records, four-year psychological examination, and seven-year neurological and psychological examinations.

To date, maternal parameters analyzed included age of gravida, parity, prior pregnancy outcome, prior and current health status, ABO blood group, current smoking pattern, and viral antibody status.

Data was used from Collaborative Study records received by PRB from the onset of the Study (January 1959) through December 1964. These records provided 112 live and stillbirth cardiac cases for study out of a population pool of approximately 38,000. Preliminary analysis indicated that there was a definite preponderance of mothers over 30 in the C-V Study group. Controlling for race, and removing cases with chromosomal aberrations, there were more white mothers in the 30 and over age group than expected at the .05 level. This trend is also noted among Negroes. There was a greater than expected number of gravida with systemic disease complications and prior pregnancy loss among the mothers of the cardiacs. A breakdown of these factors for greater specificity is pending.

Because the number of patients with each specific cardiac abnormality was small, specific associations between serological findings and clinical observations were not possible, although several interesting trends were noted.

A preliminary report on the 1964 cohort study was presented at the 1965 APHA Annual Meeting and the 1966 Annual Meeting of the Teratology Society.

Analysis of an expanded Study cohort with a population pool of approximately 55,000 providing additional cases, is underway in anticipation that additional cases will support earlier findings and perhaps provide further clues for identifying etiological relationships.

The urgent priority of processing the entire Collaborative Project study data has temporarily delayed further work on this study.

Key description words:	Congenital Heart Disease, Collaborative Perinatal Project, Epidemiological Studies, Prospective Studies-cardiac, Heart Defects-etiology
Honors and Awards:	None
Publications:	None

Project No. Z01 NS 01184 - 13 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Population Dynamics of Tay-Sachs Disease and other Sphingolipidoses

Previous Project Number: NDS (CF)-63 PR/PSC 1184

Principal Investigators: Dr. N.C. Myrianthopoulos, PRB, NINCDS

Other Investigators: Dr. D. Gröschel, University of Texas, Houston

Cooperating Units: None

Man Years:

Total:	.05
Professional:	.05
Other:	.00

Project Description:

Objectives: To confirm experimentally the epidemiologic finding that the selective advantage of the Jewish TSD heterozygote is due to possible protection of the heterozygote from tuberculosis.

Methodology: The experimental design is to measure the rate of growth of the mycobacterium tuberculosis in media with and without hexosaminidase A, and the rate of infection by the mycobacterium of tissues with and without lipid accumulation. Experiments are now in progress in which mouse macrophages are fed GM_2 ganglioside *in vivo* and *in vitro*, and the phagocytic activity for mycobacteria measured.

Major findings: None

Keyword descriptors: TSD heterozygote, selective advantage, TB resistance

Honors and Awards: None

Publications: None

Project No. Z01 NS 01274 - 11 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Genetic Bases of Neonatal Reflexes

Previous Project Number: NDS (CF)-65 PR/PSC 1274

Principal Investigators: Dr. A.F. Naylor, PRB, NINCDS

Other Investigators: Dr. N.C. Myrianthopoulos, PRB, NINCDS

Cooperating Units: None

Man Years:

Total:	.00
Professional:	.00
Other:	.00

Project Description:

Objectives: To investigate the validity of regarding the suck, rooting and other neonatal reflexes as genetic entities.

Major findings: An initial set of cases retrieved for absence of one or more of these reflexes was reviewed and seemed to have high frequencies of various kinds of trauma whose base line frequencies were unknown.

Proposed course: To place limits on the frequenices of losses of suck, rooting, palmar grasp, plantar grasp and Moro reflexes because of mutation or segregation at gene loci specifically affecting manifestation of these reflexes.

Completion of the Variable and Family Linkage Files makes practical the reactivation of this project along proper lines. Base populations can be selected for general health, especially neurological, and frequencies of isolated absence or weakness of single neurological signs can be tested. Active work on this project will be undertaken when most current tasks have been carried out.

Keyword Descriptors: Neonatal reflexes, genetics, suck, rooting, palmar, planter, Moro.

Honors and Awards: None

Publications: None

Project No. Z01 NS 01276 - 11 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Sequential Aspects of Occurrence of Spontaneous Abortion in Family Histories

Previous Project Number: Serial No. NDS (CF)-65 PR/PSC 1276

Principal Investigator: Dr. A.F. Naylor, PRB, NINCDS

Other Investigators: Dr. Dorthy Warburton, College of Physicians and Surgeons of Columbia University

Cooperating Units: None

Man Years:

Total:	.10
Professional:	.05
Other:	.05

Project Description:

Objectives: To relate the risk of spontaneous abortion to maternal age and prior reproductive experience. A special point under investigation is whether apparent age effects are explicable by a tendency for intrinsic habitual aborters to remain in the reproductive population longer in attempts to compensate for unsuccessful pregnancies. Also conditional risks have been estimated.

Proposed course: A manuscript arguing that age effects are real but laying emphasis on the decided importance of parity effects has been published. Dr. Warburton is to begin a draft of the second manuscript which will deal with conditional risks of spontaneous abortion on the basis of tables and analyses which have been in existence for some time.

Keyword descriptors: spontaneous abortion, parity, maternal age, conditional risks

Honors and Awards: None

Publications: Naylor, A.F.: Sequential aspects of spontaneous abortion: Maternal age, parity and pregnancy compensation artifact. Soc. Biol. 21: 195-204, 1974.

Project No. Z01-NS-01338-10-PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Association of Mental Subnormality with Head Circumference, Congenital Malformations, and Other Conditions of the Newborn Term Infant

Previous Project Number: NDS (CF)-66 PR/PSC-1338

Principal Investigator: Lenore Bajda, M.D., PRB, NINCDS

Other Investigators: None

Cooperating Units: Office of Biometry

Man Years:

Total:	0
Professional:	0
Other:	0

Project Description: The objective of this study is to determine the relationship between head size and certain other physical features of the Collaborative Study child noted shortly after birth, at the one-year examination and at 4 years upon completion of the psychological examination.

This study had been in abeyance pending completion of processing of all of the CPP data. In the interim a more extensive study was developed by CPP investigators which in effect incorporates most of the objectives of this study. Therefore this project is now terminated.

Honors and Awards: None

Publications: None

Project No. Z01 NS 01514 - 09 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Record Linkage of Relatives Registered in the Collaborative Study

Previous Project Number: Serial No. NDS (CF)-67 PR/PSC 1514

Principal Investigator: Dr. A.F. Naylor, PRB, NINCDS

Other Investigators: Dr. N.C. Myrianthopoulos, PRB, NINCDS

Cooperating Units: None

Man Years:

Total:	.80
Professional:	.75
Other:	.05

Project Description:

Objectives: To identify all relatives of gravidae registered in the Collaborative Project and link their records to facilitate genetic studies of obstetric, pediatric, psychological and sensory data.

Methodology: Although an existing version of the Family Linkage file was adequate for genetical obstetric studies (described in Project No. Z01 NS 01910 - 05 PR), reviews intended to correct supposedly minor flaws uncovered errors, omissions of information, and inconsistencies in format which would have made the file useless for studies in the Malformations and other programs.

Proposed course: The file will be completed and applied in familial studies of malformations, and items in other program areas.

Keyword descriptors: Record linkage, family, genetic

Honors and Awards: None

Publications: None

Project No. Z01 NS 01515 - 09 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Rh Hemolytic Disease in Negro and White Infants

Previous Project Number: NDS (CF)-67 PR/PSC 1515

Principal Investigators: A.F. Naylor, PRB, NINCDS

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.00
Professional:	.00
Other:	.00

Project Description:

Objectives: To confirm a report that high Rh antibody levels have smaller morbid effects in Negro than in white babies, although this is not true for ABO antibodies.

Major findings: Preliminary and indirect confirmation has been obtained, from a small data sample under study in this Section, for reports in the literature that high Rh antibody titers are not as highly associated with serious morbidity in Negroes as in whites.

Proposed course: The existence of the Variable Data File will make possible the easy execution of the required data processing.

Keyword Descriptors: Hemolytic disease, Rh, ABO, Negro, white.

Honors and Awards: None

Publications: None

Project No. Z01 NS 01516 - 09 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Size of Placenta in Relation to Mother-Fetus Antigenic Difference

Previous Project Number: NDS (CF)-67 PR/PSC 1516

Principal Investigators: Dr. Dorothy Warburton, College of Physicians and Surgeons of Columbia University
Dr. A.F. Naylor, PRB, NINCDS

Other Investigators: Dr. G. Nicholas Rogentine, Immunology Branch, NCI
Dr. Robert Cefalo, Obstetrics, National Naval Medical Center

Cooperating Units: Obstetrics Department, National Naval Medical Center

Man Years:

Total:	.00
Professional:	.00
Other:	.00

Project Description:

Objectives: To investigate the hypothesis published by Billington in 1964, that sensitization of the mother during pregnancy against paternal antigens leads to non-pathological placental hypertrophy and increased birthweight in succeeding pregnancies.

Major findings: Analysis of a large sample of study data has given convincing support to the hypothesis. A paper reporting the evidence has been published.

Proposed course: The arrangements for a laboratory study, described in the 1969-70 Annual Report have been active and 44 maternal sera have been tested against paternal leucocytes. Logistic complications which cause poor data return for effort have stopped collection of new cases. The accumulated data must be reviewed for publishability, but abandonment seems likely.

Keyword Descriptors: Placenta, birthweight, antigens

Honors and Awards: None

Publications: None

Project No. Z01 NS 01754 - 07 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Growth and Intellectual Development of Children from Interracial Matings

Previous Project Number: Serial No. NDS (CF)-69 PR/PSC 1754

Principal Investigator: Dr. Lee Willerman, University of Texas at Austin

Other Investigators: Dr. Alfred F. Naylor, PRB, NINCDS
Dr. Ntinos C. Myrianthopoulos, PRB, NINCDS

Cooperating Units: Department of Psychology, The University of Texas at Austin

Man Years:

Total:	.05
Professional:	.05
Other:	.00

Project Description:

Objectives: Results of analyses of growth and intellectual development have been prepared for separate publication.

The conclusions reached in an initial publication in Science, that analyses of CPP interracial for IQ data indicate that race of mother as a postnatal environmental indicator accounts for much of the black-white IQ differences, have been strengthened in a paper published this year. Analyses of Bayley Mental and Motor Scores, taken at 8 months, when maternal social influences will have had little effect show no differences between children of black/white and white/black matings. Also a more convincing demonstration has been made of lack of bias in genetic or socio-economic factors affecting four year IQ on the paternal side.

The physical development data have been re-analyzed to compare all properly selected CPP interracial and monoracial matings. Hospital variation and other background factors were corrected for by multivariate regression. At birth children born to white mothers, whether by white or black fathers, are very similar in weight and length. Monoracial blacks are definitely smaller and interracials with black mothers may be intermediate in size (small numbers cloud the issue). At four months interracials with white mothers fall behind in weight (but catch up after one year), perhaps because of social stresses in the household. A paper has been submitted for publication.j

Keyword descriptors: interracial, black, white, IQ, growth, body weight, length

Honors and Awards: None

Publications: None

Project No. Z01 NS 01857 - 06 PR
1. Perinatal Research Branch
2. Professional Staff Consultants
Section
Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Genetics of Intellectual and Motor Performance

Previous Project Number: NDS (CF)-70 PR/PSC 1857

Principal Investigators: Sarah H. Broman, Ph.D., PRB, NINCDS
Paul L. Nichols, Ph.D., PRB, NINCDS

Cooperating Units: None

Man Years:

Total: .04
Professional: .04

Project Description:

Objectives: The objectives of the study are to assess the contribution of genetics to the variance of behavioral measures, particularly intellectual and motor performance, by correlating scores and measurements of twin, sibling and half-sibling pairs. A report of twin and sibling correlations on the eight-month Bayley Mental Scale has been published. Analyses of four and seven year data are continuing.

Keyword Descriptors: Intellectual performance, IQ, genetics, Twins heritability.

Honors and Awards: None

Publications:

Nichols, P. L. and Anderson, V. E.: Intellectual performance, race and socioeconomic status. Soc. Biol., 20: 367-374, 1973.

Nichols, P. L. and Broman, S. H.: Familial resemblance in infant mental development. Dev. Psychol., 10: 442-446, 1974.

Project No. Z01 NS 01904-05 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Study of Labor and Delivery
Previous Project No. NDS (CR) 71 PR/PSC 1904

Principal Investigator: Primarily a Task Force Investigation
Dr. Vincent Tricomi, Chairman

Other Investigators: Emanuel A. Friedman, M.D., Harvard University Medical School, Boston, Massachusetts
Luke Gillespie, M.D., Boston Lying-In Hospital, Boston, Massachusetts
Marvin Green, M.D., New York Medical College, New York, New York
Esther Jackson, Office of Biometry, NINCDS
Schuyler G. Kohl, M.D., State University of New York, Downstate Medical Center, Brooklyn, New York
Kenneth R. Niswander, M.D., University of California at Davis, California
David Rubinstein, Office of Biometry, NINCDS
Vincent Tricomi, M.D., Brooklyn-Cumberland Medical Center, Brooklyn, New York

Cooperating Units: All institutions participating in the Collaborative Project

This project has been discontinued. Upon completion of the current analysis consideration will be given to initiating a new study in this area.

Project No. Z01 NS 01910 - 05 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Genetics of Obstetric Variables and the Role of Maternal Factors in the Determination of Intelligence and Neurological Performance

Previous Project Number: NDS (CF)-71 PR/PSC 1910

Principal Investigators: Dr. A.F. Naylor, PRB, NINCDS
Dr. N.C. Myrianthopoulos, PRB, NINCDS

Other Investigators: Dr. D. Warburton, College of Physicians and Surgeons of Columbia University

Cooperating Units: Department of Human Genetics and Development, Columbia University

Man Years:

Total:	.10
Professional:	.05
Other:	.05

Project Descrition:

Objectives: To analyze the variation of obstetric and gynecological factors into heritable and non-heritable components.

Major findings: The completed Record Linkage files have been used to estimate genetic components in obstetric variables, both continuous and discrete: age at menarche, lengths of stages of labor, gestation time, diagonal conjugate, anemia, toxemia, albuminuria, hemorrhage, abnormal presentation, abruptio placentae, fetal death and nine others. A quite substantial paper, which seems to be the first systematic discussion based on directly relevant data, has been published.

Keyword Descriptors: Obstetric, genetic, menarche, gestation, labor, toxemia, anemia, fetal death.

Honors and Awards: None

Publications:

Naylor, A. F. and Warburton, D.: Genetics of obstetrical variables. A study from the collaborative perinatal project. Clin. Genet. 6:351-369, 1974.

Project No. Z01 NS 01913 - 05 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Clinical Significance of Generalized Petechiae at Birth

Previous Project Number: NDS (CF)-71 PR/PSC 1913

Principal Investigators: Luz A Froehlich, M.D., NIAID
Jean G. Oliver, M.A., PRB,NINCDS

Other Investigators: Jean S. Nemore,R.N., B.A., PRB,NINCDS

Cooperating Units: All Collaborating Institutions

Man Years:

Total:	0.0
Professional:	0.0
Others:	0.0

Current Status:

Evaluation of selected variables did not show any significant differences between cases with significant petechiae and matched controls with respect to speech, language and hearing findings. Therefore, this project has been terminated.

Honors and Awards: None

Publications: None

Project No. Z01 NS 01968 - 04 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Preschool IQ: Prenatal and Early Developmental Correlates

Previous Project Number: NDS (CF)-72 PR/PSC 1968

Principal Investigators: Sarah H. Broman, Ph.D., PRB,NINCDS
Paul L. Nichols, Ph.D., PRB,NINCDS
Wallace A. Kennedy, Ph.D., Florida State University

Cooperating Unit: Florida State University

Man Years:

Total:	.8
Professional	.5
Other:	.3

Project Description:

Objectives: The relationship between intelligence at age four as measured by the Stanford-Binet Intelligence Scale and a variety of factors related to pregnancy, delivery, postnatal status and social environment was examined among 26,760 white and Negro childrn enrolled in the Collaborative Perinatal Project.

Method: The plan of analysis of these data was exploratory, beginning with an identification of antecedents related to IQ. The results of a variable screen in which 169 prenatal, postnatal and environmental factors were correlated with IQ are presented. The 83 variables found to be significantly related to IQ are defined and their distributions in the sample are given. Mean IQs for different levels of each predictor variable are displayed within race, sex, and social class controls. Significant main effects and interactions are discussed. The combined effects of the antecedent variables were examined in a multiple regression model, and also in a discriminant function model with subjects divided into low and normal IQ groups. Methodological issues discussed include the selection of an outcome variable at four years, the problem of non-linear relationships between antecedent variables and IQ, and the effect of combining data from different study centers.

Major Findings: All predictor variables accounted for only 25 to 28 percent of the variance in IQ among whites and 15 to 17 percent among Negroes. The prenatal variables alone accounted for 20 percent of the IQ variance among whites, and eight to 11 percent among Negroes, with maternal education and the socioeconomic index contributing the largest proportion of explained variance not only in this first analysis but also when neonatal and childhood variables were added. The estimated effects of maternal education and the SEI on IQ were greater in the white than in the Negro samples. The neonatal variables accounted for only an additional one to two percent of the variance, while the infancy and childhood variables added four to six percent.

Prior to birth the most consistent predictor of mental retardation at age four was maternal education. When variables from the neonatal period were included a diagnosis of brain abnormality, size of the neonate (head circumference or length), maternal education and socioeconomic index of the family were significantly associated with retardation. After status during the first year of life and size at four years were considered, the factors most highly related to retardation were delayed motor development at one year, mental and motor test scores at eight months, maternal education, and relatively rare conditions associated with neurological impairment during the first year of life.

Proposed Course: This study has been completed.

Keyword Descriptors: IQ, intelligence, mental retardation, prenatal factors, physical measures, infant developmental tests, socioeconomic status

Honors and Awards: None

Publications:

Broman, S.H., Nichols, P.L., and Kennedy, W.A.: Preschool IQ: Prenatal and Early Developmental Correlates. Hillsdale, N.J.: Lawrence Erlbaum Associates (distributed by Halsted Press of John Wiley & Sons) 1975, 360 pp.

Project No. Z01 NS 01971 - 04 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biosocial Factors Associated with Communicative Disorders and Competence in Children

Previous Project Number: NDS (CF)-72 PR/PSC 1971

During FY 1975 this project was incorporated with Contract Narrative Number: N01-NS-4-2326.

Project No. Z01 NS 01972 - 04 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Relationships Between Spontaneous Speech and Other Test Results at Eight Years of Age

Previous Project Number: NDS (CF)-72 PR/PSC 1972

Principal Investigators: Jean G. Oliver, M.A., PRB-NINCDS

Other Investigators: Paul J. LaBenz, Sc.D., PRB-NINCDS

Cooperating Units: All Collaborating Institutions

Man Years:

Total:	0.0
Professional:	0.0
Others:	0.0

Project Description:

The study of spontaneous speech can provide useful insights into communicative skills, and appears to be a valid index of expressive language proficiency. Samples of spontaneous speech, elicited by visual stimuli (pictures) are being tape-recorded opportunistically at quality control visits where 8-year old children are retested. These samples are later transcribed for analysis. Performances are rated for comparison with data derived from the standard speech, language and hearing battery.

Method:

A tentative set of rating scales has been devised to enable evaluation of several aspects of spontaneous speech output. These include the length of the speech sample as well as the content in terms of quality, organization, elaboration and grammatical structure. Relationships between spontaneous connected speech and a number of traditional measures of communicative ability are to be explored using correlation methods.

Current Status:

This project has been temporarily suspended until the basic Comprehensive Plan is further along to permit time for this special study.

Keyword Descriptors: Spontaneous speech, expressive language proficiency

Honors and Awards: None

Publications: None

Project No. Z01 NS 02052-03 PR
1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The First Year of Life

Previous Project Number: NDS (CF)-73 PR/OC 2052

Principal Investigators: Joseph S. Drage, M.D., Chief, Perinatal Research Branch, C&FR, NINCDS
Janet B. Hardy, M.D., Associate Professor of Pediatrics, The Johns Hopkins University
Esther Jackson, Head, Section on Applied Statistics, Office of Biometry, C&FR, NINCDS

Other Investigators: None

Cooperating Units: The Johns Hopkins University; Perinatal Research Branch, C&FR, NINCDS; Office of Biometry, C&FR, NINCDS

Man Years:

Total:	.2
Professional:	.1
Other:	.1

Project Description:

Objectives: "The First Year of Life" is planned as a volume to report on the frequency distribution of a number of findings reported on Collaborative Perinatal Project children during the first year of their lives. It will include information on birthweight-gestation distributions, bilirubin levels, age at hospital discharge, and distributions of various pathological findings detected during the nursery stay and during the first year of life. Of particular interest will be information regarding brain abnormality as detected during the nursery period. This volume is intended to serve as a general description of the Collaborative Perinatal Project children during their first year of life and as a reference document for further in-depth studies. Work has continued on this project. Most of the tables, charts, and chapters are completed. A manuscript should be available for outside review by early fall, 1975.

Project Descriptors: neonate, infancy, birthweight-gestation, bilirubin levels, brain abnormality, nursery, perinatal

Honors and Awards: None

Publications: None

Project No. Z01-NS-02053-03-PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Natural History of Congenital Toxoplasmosis: Case Studies from the Collaborative Perinatal Research Project

Previous Project Number: NDS (CF)-73 PR/PSC-2053

Principal Investigator: Lenore Bajda, M.D., PRB, NINCDS

Other Investigators: Dr. John L. Sever, ID, NINCDS
M. R. Gilkeson, ID, NINCDS

Cooperating Units: Infectious Diseases Branch, NINCDS

Man Years:

Total:	.50
Professional:	.50
Other:	.00

Project Description:

Objectives: 1) A search of CPP data for clues serving as indicators for degree of fetal risk to maternal Toxoplasma infection 2) To contribute to the prevention of Congenital Toxoplasmosis thru an elucidation of its natural history in the CPP population.

Methods employed: A subsample of 47 gravidae with markedly elevated titers or four-fold rises in Toxo antibody and their 94 control gravidae was provided from an initial CPP population of 23,000 gravidae in the study from onset thru March, 1963 (Sever, J. L. Toxoplasmosis: Serological and Clinical Studies -- Serial No. NDS (CF)-65 PR/ID 1270). This subgroup was subjected to an intensive review of all pertinent original protocols in CPP central files. Maternal characteristics particularly examined in addition to age, race and socio-economic background were prior pregnancy failures, past medical, and current health histories (including drug therapy). Pediatric data reviewed included all medical information available thru the 7 year Neurology examination as well as global IQ scores of the 4 and 7 year Psychological examinations.

Major Findings: Maternal findings included a) a constant exposure to Toxoplasmosis throughout the child-bearing years, b) higher exposures among Negroes and women born in Puerto Rico, and c) no correlation with urban vs. rural life. Additional findings requiring further support included prior pregnancy failures within 1 year to LMP being greater than in the controls (8.5 to 3.2%) and the observation that a frequent use of sulfa drugs in the treatment of urinary tract infection during the pregnancy (17%) may have a significant effect on the spectrum of pregnancy outcome. Pediatric findings at a seven year endpoint added to the previously published one-year findings the additional observations of a high rate of "undefined" abnormalities in the case group compared to controls and a high rate of low IQs (24 compared to 17%). The latter finding may become less significant on adjustment for birthweight and degree of maternal education.

The implication of the as yet unknown and difficult to measure effect of drug treatment for coexistant medical problems in the Toxoplasma-infected mother demands further study. The spectrum of congenital infection has been expanded to include the possibility of "learning dysfunction" or "disability" in an otherwise "normal" child. This challenge needs urgent attention particularly since Congenital Toxoplasmosis is a preventable disease.

The results of this project were presented at a Symposium on Toxoplasmosis during the XIIIth Congress of the Medical Women's International Association, Paris, France, September 3-8, 1972. Publication is pending.

Key Word Descriptors: toxoplasmosis, perinatal infections, congenital infections.

Honors and Awards: None

Publications: None

Project No. Z01 NS 02058 - 03 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Convulsive Disorders Data Analysis Group

Previous Project Number: NDS (CF)-73 PR/PSC 2058

Principal Investigators: Karin B. Nelson, M.D., PRB, NINCDS
Jonas Ellenberg, Ph.D., OB, NINCDS

Other Investigators: John Freeman, M.D., Johns Hopkins
Kenton Holden, M.D., Johns Hopkins

Cooperating Units: None

Man Years:

Total	3.1
Professional:	1.4
Other:	1.7

Project Description:

Objectives: We will examine maternal characteristics, conditions of pregnancy, labor, delivery and the neonatal period, and illness and injuries of early childhood for their association with seizure disorders. Our aim is to identify points at which preventive efforts could usefully be directed, to seek clinically useful indices of prediction, to evaluate clustering of other handicaps with convulsive disorders, and to examine the frequency of seizure disorders in the population of the Collaborative Perinatal Project.

Methodology: Case review has been completed on approximately 2700 records of children thought to have experienced convulsive disorders. This clinical material has been classified and coded, and is now being prepared for file creation, and thus readied for analysis. An extensive new pediatric variable file has been prepared and tested, taking special account of early neurological findings and, where possible, reconciling inconsistencies in form revisions. The first phase of programming has been written and tested to examine demographic characteristics of children with seizure diagnoses, and we will then undertake a univariate screen of the association between seizure diagnoses and antecedent characteristics of the mother, of obstetrical events, the neonatal course, and intervening illnesses and injuries.

Drs. Freeman and Holden are pariticipating in the study of neonatal seizure states.

Major Findings: No analyses have yet been completed on the data on convulsive disorders.

Future Course: When the case file has been completed, demographic analyses and the univariate screen of antecedent obstetric and pediatric characteristics will be undertaken. The most significant risk factors identified in the univariate screen will then be subjected to multivariate analysis to assess the independent contribution of each. In addition, a number of specific questions will be asked of this data, having to do with the recurrence of seizure disorders, the occurrence of later nonfebrile seizure disorders in children whose initial seizures were febrile, the outcomes in motor and intellectual terms of children with various early seizure diagnoses, etc.

Significance: The convulsive disorders are a common and socially costly medical problem. It is estmated that about 4 million Americans have some form of epilepsy. The cost of the epilepsies in direct payments and medical expenses has been calculated at more than $4 billion per year. The information generated in the Collaborative Perinatal Project may significantly influence medical and social policy decisions having to do with this important problem area.

Keyword Descriptors: Convulsive disorders, epilepsy, seizures

Honors and Awards: None

Publications: None

Project No. Z01 NS 02059 - 03 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cerebral Palsy Data Analysis Group

Previous Project Number: NDS (CF)-73 PR/PSC 2059

Principal Investigators: Karin B. Nelson, M.D., PRB, NINCDS
Jonas Ellenberg, Ph.D., OB, NINCDS

Other Investigators: Alan Leviton, M.D., Children's Medical Center, Boston, Mass.

Cooperating Units: None

Man Years:

Total:	3.5
Professional:	1.6
Other:	1.9

Project Description:

Objectives: We seek to examine the etiology of motor disabilities in children, to improve clinical prediction, and to examine the relative frequencies of motor and associated disabilities in the population of the Collaborative Perinatal Project.

Methodology: Through hand review of approximately 850 clinical records, and classification according to a consistent system, we have identified children with cerebral palsy among those examined at one and seven years.

A new and extensive file of pediatric variables has been created and tested, reconciling where possible inconsistencies in form revisions. The first phase of computer programming has been written and tested to permit screening of antecedent obstetric variables and early clinical manifestations with regard to their association with cerebral palsy diagnoses.

Major Findings: Only results of hand tabulations are as yet available. Children with mild, early CP often lose their motor deficits by school age, although they remain at heightened risk for intellectual subnormality. Children with severe early CP usually retain their motor handicaps, or die with CP. Some clinically useful predictors of status at school age have been identified.

Future Course: It is expected that machine tabulations with respect to demographic analyses and the univariate screen of antecedent obstetric and pediatric characteristics will be completed before the end of this fiscal year. The most significant risk factors identified in the univariate screen will then be subjected to multivariate analysis, to assess the independent contribution of each.

Significance: Approximately 750,000 persons in the United States are victims of cerebral palsy; milder forms of CP are more frequent still. As these individuals are afflicted from earliest childhood, the loss in social and economic terms is immense. Many are lifelong dependents of their families or the state. Our aims are to identify areas in which preventive efforts may be effectively directed, to improve clinical prognostication, to examine clustering of handicaps, and to estimate relative frequency of CP conditions. Information in these areas may help to indicate fruitful directions for both medical and social policy decisions.

Keyword Descriptors: Cerebral palsy, obstetrics, mental retardation

Honors and Awards: None

Publications: None

Project No. Z01 NS 02060 - 03 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Birthweight-Gestational Age

Previous Project Number: NDS (CF)-73 PR/PSC 2060

Principal Investigators: Dr. Bill H. Williams, PRB, NINCDS

Other Investigators:
Dr. Janet Hardy, Johns Hopkins
Dr. Joseph S. Drage, PRB, NINCDS
Dr. Kinne D. McCabe, PRB, NINCDS
Ms. Esther Jackson, OB, NINCDS
Mr. William Weiss, OB, NINCDS
Dr. E. David Mellits, Johns Hopkins

Cooperating Units: Johns Hopkins University

Man Years:

Total:	0.50
Professional:	0.25
Other:	0.25

Project Description:

During the past year through the use of the polynomial regression program, the relationships of birthweight to secondary parameters such as placental weight, maternal education, socioeconomic index, gestation at registration, year of birth, institution, body length at birth and head circumference at birth have been examined. A covariance program has been developed to study particularly the relationship of birthweight to placental weight and to gestational age, for survivors and for non-survivors. A multivariant analysis will be pursued to determine the relationship of these variables to antecedent factors (prenatal) and later outcomes (particularly neurological development). Empirical Risk Tables for babies in certain birth-weight-gestational age categories will be developed and a Birthweight Index will be generated which will be of some predictive value in relating antecedent factors to birth-weight-gestational age outcomes. Plans are underway to incorporate the entire body of information generated in the form of a monograph which would be of use to clinicians.

Keyword Descriptors: polynomial regression, birthweight, placental weight, maternal education, socioeconomic index, gestation at registration, year of birth, institution, body length at birth, head circumference at birth, covariance program, birthweight to placental weight, birthweight to gesta-

tional age, survivors, non-survivors, multivariant analysis, relationship of variables to antecedent factors, neurological development.

Honors and Awards: None

Publications: None

Project No. Z01 NS 02062 - 03 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Minimal Brain Dysfunction

Previous Project Number: NDS (CF)-73 PR/PSC 2062

Principal Investigator: Paul L. Nichols, Ph.D., PRB, NINCDS

Other Investigators: Ta-chaun Chen, Ph.D., OB, NINCDS

Cooperating Units: None

Man Years:

Total:	.75
Professional	.65
Others:	.10

Project Description:

Specific diagnoses of minimal brain dysfunction have not been made for children in the longitudinal Collaborative Perinatal Project, but there is information available related to the most frequently cited symptoms (e.g., hyperactivity, learning difficulties, and equivocal neurological signs). This study will attempt to develop MBD criteria from the available CPP data, characterize MBD children in terms of demographic, psychological, and physical variables, and relate MBD or its components or individual symptoms to socioeconomic, maternal, neonatal, perinatal, and other antecedent variables.

Antecedent variables to be investigated will be selected from among family characteristics, maternal characteristics, the prenatal period, labor and delivery variables, the neonatal period, infancy and childhood characteristics, and intercurrent events. It is expected that a minimum of 300 antecedent variables will be examined. The selection and definition of many of these variables will be in collaboration with investigators in other primary areas.

Based on test data already received, the following preliminary observations are possible: (1) The risk of problems in one area is increased between

40% and 100% if a child has problems in one of the other four areas. That is, there is a significant association among the symptoms in the four areas. (2) Most children, however, who have problems in one area (e.g., physical) do not have problems in the other areas (e.g., behavioral or academic). In other words, the association among symptoms is not a strong one. (3) Correlations and factor analyses of the individual symptoms have identified four major factors that will be studied in detail. These include academic problems, minor neurological problems, hyperactivity-impulsivity, and "socio-emotional immaturity."

Keyword Descriptors: Minimal brain dysfunction, learning disabilities, hyperactivity, neurological soft signs, longitudinal study.

Honors and Awards: None

Publications: None

Project No. Z01 NS 02106 - 02 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Developmental Factors Associated with Mental Retardation at Age Seven

Previous Project Number: NDS (CF)-74 PR/PSC 2106

Principal Investigator: Sarah H. Broman, Ph.D., PRB,NINCDS

Other Investigator: Paul L. Nichols, Ph.D., PRB, NINCDS

Cooperating Unit: None

Man Years:

Total	0.7
Professional	0.5
Other	0.2

Project Description:

Objectives: Mental retardation is a serious social problem. Approximately three percent of the population is affected according to the conventional criterion of intelligence test scores under 70. Data collected in the CPP are being analysed to determine the primary and contributing roles of biological, environmental and familial factors in mental retardation in a biracial population of 40,000 seven year old children followed since the prenatal period. In this population, approximately two percent of the white sample and five percent of the Negro sample have IQs under 70. The identification of early signs of mental retardation will facilitate prevention, diagnosis and treatment, and will add substantially to knowledge in this area which has been largely derived from small retrospective studies of institutionalized retardates. The objectives of this study are to determine the degree of association between mental retardation at age seven and the following classes of variables:

1) Biological factors

   a) Complications of pregnancy and delivery
   b) Adverse neonatal conditions

- c) Neurological and general medical status at one and seven years
- d) Childhood diseases and accidents
- e) Physical growth rates

2) Socio-environmental factors

- a) Socioeconomic status and social mobility of family
- b) Parental education
- c) Family composition
- d) Geographic area of residence

3) Familial factors: Occurrence of retardation in relatives

- a) Monozygotic and dizygotic twins
- b) Full and half-sibs
- c) Other relatives

4) Cognitive development

- a) Mental and motor development at eight months
- b) IQ scores, fine and gross motor development and conceptual development at age four
- c) Conceptual, visual-motor and behavioral development at age seven

Method: In this study, mental retardation is defined as an IQ of 70 or less on the Weschler Intelligence Scale for Children, or, for the relatively few children who could not be tested according to study protocol, equivalent IQs from other tests or reliable clinical judgements of retardation. This latter source of classification applies mainly to severely retarded children who have been institutionalized.

Since children with IQs under 70 form a heterogeneous group, they will be subdivided into four major categories consisting of those with severe retardation (IQ under 50) with and without signs of central nervous system damage, and those with mild retardation (IQ between 50 and 69) with and without such signs. Specific neurological diagnoses, such as cerebral spastic paresis, seizure states, Down's syndrome (mongolism) and head trauma, will be obtained from the neurological examination given at age seven. The four groups will be further subdivided by ethnicity (white, Negro and Puerto Rican) and, where the number of cases is sufficient, by sex. Comparison groups will be composed of children with IQs in the borderline, average and superior ranges. These groups will also be checked for signs of central nervous system damage and will be subdivided by ethnicity and sex. Factors that are associated with performance in the average and superior groups, and are most useful in differentiating these groups from the mentally retarded, will be examined.

Major Findings: Preliminary findings indicate that the following conditions are related to a low level of intellectual functioning at age seven: Low socioeconomic status of the family which includes income, and occupation and education of the parents, high housing density, low maternal intelligence test scores, less prenatal care, more complications in the neonatal period, less advanced mental and motor development at 8 months, more frequent signs of central nervous system involvement at one year, poorer language development at age three, lower psychometric test performance at age four, and smaller physical measurements during the first 7 years of life.

Proposed Course: This study is scheduled for completion in June, 1976. A paper has been prepared for presentation which reports on the relationship between early mental development, social class and intellectual level at age 7.

Keyword Descriptors: Mental retardation, infant development, social class, neonatal complications, neurological abnormalities

Honors and Awards: None

Publications: None

Project No. Z01 NS 02107 - 02 PR
1. Perinatal Research Branch
2. Professional Staff Consultant Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The study of visual abnormalities in the Collaborative Study

Previous Project Number: NDS (CF)-74 PR/PSC 2107

Principal Investigator: Dr. Richard Feinberg, PRB, NINCDS

Other Investigators: Ms. Esther Jackson, OB, NINCDS
Richard J. Apell, O.D., Gesell Institute of Child Development, New Haven, Connecticut
William R. Baldwin, O.D., Ph.D., Massachusetts College of Optometry, Boston, Massachusetts
Richard E. Hoover, M.D., Baltimore, Maryland
Robert P. Kling, M.D., Georgetown University Hospital, Washington, D. C.
Milton A. Whitcomb, Ph.D., National Academy of Sciences, Washington, D. C.
Sheila Z. Wood, O.D., Washington, D. C.
Francis A. Young, Ph.D., Washington State University, Pullman, Washington

Cooperating Units: None

Man Years:

Total:	1.2
Professional:	1.2
Others:	0.2

Project Description

Objectives: To determine the extent to which genetic, maternal, obstetric, pediatric and environmental factors produce eye and visual abnormalities in the Study children; to assess the relative frequency of such anomalies; and, to study the concomitance of visual abnormalities with other sensory and motor neurological and systemic disorders.

Methods Employed: A reference cohort of visual defects is defined for which the specified outcome will be tabulated. Outcome variables have been selected. Initial computer printouts have been obtained and are in process of being analyzed.

Major Findings: At this stage, no major findings can be reported.

Proposed Course: This will include: the analysis between visual abnormalities and outcome variables; anecdotal treatment based on case histories of unusual visual abnormalities; special studies of high-incidence disorders and of concordance in siblings and twins.

Key Word Descriptors: Congenital eye defects; developmental vision; ocular dyscrasias; visual defects; visual anomalies; perceptual disorders; pediatric ophthalmology; pediatric optometry; refractive problems; visual abnormalities.

Honors and Awards: None

Publications: None

Project No. Z01 NS 02108 02 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Developmental Factors Associated with Learning Disorders at Age Seven

Previous Project Number: NDS (CF)-74 PR/PSC 2108

Principal Investigator: Sarah H. Broman, Ph.D., PRB, NINCDS

Other Investigator: Paul L. Nichols, Ph.D., PRB, NINCDS

Cooperating Unit: None

Man Years:

Total	.7
Professional	.5
Other	.2

Project Description:

Objectives: Children with normal intelligence and poor school performance, particularly in reading, present significant problems in etiology and the development of effective remedial techniques. Longitudinal data collected in the CPP on a biracial population of 40,000 seven year old children permit a study to be made of early events, beginning in the prenatal period, which differentiate between children with learning disorders in the first and second grades and those without a significant discrepancy between intellectual ability and school performance. The accurate identification of precursors of behavior patterns identified as learning disorders will facilitate prevention, early diagnosis and treatment. The objectives of this study are to determine the degree of association between learning disorders at age seven and the following classes of variables:

1) Biological factors

   a) Complications of pregnancy and delivery
   b) Maternal drug intake during pregnancy
   c) Adverse neonatal conditions and early abnormal neurological signs
   d) Neurological and general medical status at one and seven years

e) Childhood diseases and accidents
f) Physical growth rates

2) Socio-environmental factors

a) Socioeconomic status and social mobility of family
b) Parental education
c) Maternal intelligence level
d) Family size and composition
e) Geographic area of residence

3) Familial factors: occurrence of learning disorders in relatives

a) Monozygotic and dizygotic twins
b) Full and half-sibs

4) Cognitive and behavioral factors

a) Mental and motor development and behavior ratings at eight months
b) IQ scores, fine and gross motor development, concept formation and behavior ratings at age four
c) Verbal and performance IQ scores, conceptual and visual-motor development and behavior ratings at age seven

Method: In this study, a child is considered to have a learning disorder if he has an IQ of 90 or above on the Weschler Intelligence Scale for children and is more than one year below grade expectancy (actual grade placement) in reading or spelling on the Wide Range Achievement Test. Children with learning disorders will be compared with average and above-average achievers in order to isolate early signs and current characteristics. Comparisons will be made within ethnic (white, Negro and Puerto Rican), sex and social class subgroups. It is recognized that this study is limited by the absence of any follow-up on the academic performance of the CPP children beyond age seven.

Major Findings; Preliminary findings indicate that when compared with control groups matched for IQ, children with learning disorders had families of lower socio-economic status as indicated by parental education, occupation and income. Mothers of children in the learning disorder groups were of higher parity, had received less prenatal care and were more frequently unmarried. The children themselves were more frequently male, and among Negroes, tended to be smaller than controls during early childhood. Speech was less well developed at age three and psychological test scores, including IQ, were lower at age four. Hyperactivity and shorter attention span, as well as less verbal communication were also evident at age four. Similar findings emerged at age 7 even though the learning disorder groups and their controls were matched for IQ at this age.

Proposed Course: This study is scheduled for completion in June of 1976.

Keyword Descriptors: Learning disorders, IQ, socioeconomic status, early development

Honors and Awards: None

Publications: None

Project No. Z01 NS 02109 - 02 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Comprehensive Analysis and Interpretation of the Collaborative Perinatal Project Data on Congenital Malformations

Previous Project Number: Serial No. NDS (CF)-74 PR/PSC 2109

Principal Investigators: Dr. N.C. Myrianthopoulos, PRB, NINCDS

Other Investigators: Dr. A.F. Naylor, PRB, NINCDS
Mr. David Rubinstein, OB, NINCDS
Dr. C.S. Chung, University of Hawaii
Dr. Herbert Lubs, University of Colorado
Dr. Marie-Louise Lubs, University of Colorado
Dr. David Smith, University of Washington
Dr. Jaime Frias, University of Florida

Cooperating Units: Office of Biometry, NINCDS
Department of Pediatrics, University of Colorado
Department of Public Health, University of Hawaii
Department of Pediatrics, University of Washington
Department of Pediatrics, University of Florida

Man Years:

Total:	2.60
Professional:	2.00
Other:	0.60

Project Description:

Objectives: To thoroughly study the epidemiologic characteristics of congenital malformations in singletons and twins; to assess and interpret the influence of maternal, socioeconomic, neonatal, medical and other environmental factors on the occurrence of congenital malformations; to determine the risk of familial occurrence and to elucidate the role of genetic factors and the mode of inheritance of certain malformations; to determine the severity and clinical significance of congenital malformations and their associations with neurological, psychological and sensory handicaps; and to assess the long-range effects of malformations on survival, growth and development.

Methodology: A congenital malformation is defined as a gross physical or anatomical developmental anomaly which was present at birth or was detected during the first year of life. Malformations have been classified into major and minor categories on the basis of their severity, threat to life and cosmetic significance.

The analysis is divided in 11 parts, and for each appropriate epidemiologic and statistical methods are being employed.

Current status and major findings:

I. Epidemiology of congenital malformations in singletons.

This part has been completed. About 15 percent of single-born children had one or more malformations. Half of the malformations were major. Only about a third of malformations observed during the first year of life were diagnosed at birth. Except for three minor malformations which were more frequent among Negroes, there were no significant differences in malformation incidences between Negroes and whites.

A report of this part has been published.

II. Epidemiology of congenital malformations in twins.

This part has been completed. Twins have significantly more major and minor malformations than singletons but the difference is wholly contributed by monozygotic (MZ) twins. The incidence of malformations in dizygotic twins is the same as that in singletons. Monoamniotic twins have more malformations than diamniotic twins. Concordance rates are significantly higher among MZ than among DZ twins for all malformation categories but among specific malformations only those of the musculoskeletal system show significant differences.

A report has been prepared and is now in press.

III. A study of the effects of medical, genetic and socioeconomic factors in the occurrence of congenital malformations.

The analysis of this part has been completed. Multiple birth, pregnancy complications (mostly through hydramnios) and male birth were positively correlated with increased risk in major malformations, whereas maternal weight gain was negatively correlated with major malformations. Maternal diabetes during pregnancy was significantly correlated with single or multiple major malformations. This finding is further explored in Part IV.

A report has been written and is now in press.

Over one-third of children of chronic alcoholic mothers show a constellation of developmental deficits and clinical symptoms known as the "fetal alcohol syndrome". A report has been published.

An analysis of malformations in children of mothers who took dilantin during pregnancy is now in progress.

IV. Special analysis of the effects of diabetes in the mother on the occurrence of congenital malformations in the offspring.

The analysis for this part has been completed. The risk of having a malformed child in mothers with continuous diabetes is doubled compared to that of non-diabetic mothers, with regard to major malformations and increased significantly with regard to minor malformations. The effect seems to be associated with the severity of the disease and not with the intake of insulin.

A report is now being written.

V. Genetic studies of congenital malformations.

This part is in progress. The studies have been designed to derive empiric risk figures of repeating a malformation when it has once appeared in a family; to identify familial aggregations of specific malformations; and to clarify the mode of inheritance in identified familial aggregations.

Special files required for the analysis have been generated. These include files of study siblings, half siblings and twins. Another file containing all relatives of gravidae registered in the Collaborative Project is being completed. Over 3,000 mutually exclusive family groups have been identified and family linkage relationships have been established.

VI. Study of associations of ABO and Rh blood types with congenital malformations.

This study has been designed to confirm earlier suggestions of association of blood groups with congenital malformations, based on small samples.

The study is now in progress. Several strong associations have been detected and special analyses are being performed to identify random and non-random associations.

A report is being written.

VII. Study of the clinical significance of minor malformations.

VIII. Longitudinal study of development, morbidity and survival of children with malformations.

These two studies have been designed in collaboration with Dr. David Smith of the University of Seattle, Washington, and Dr. C.S. Chung of the University of Hawaii. The objective of Part VII is to establish which minor malformations are worthwhile detecting and why, and which ones can be ignored or considered as normal variants. Part VIII is a continuation of Part VII and deals with the effects of single and multiple malformations on growth and development.

All cases with multiple malformations are now being reviewed. Preliminary analysis of the first 212 cases showed that 14% represent known malformations syndromes, 54% primary single malformations with accompanying secondary malformations, and 32% remain unclassified, probably a mixture of unknown syndromes and random associations.

IX. Study of the effects of maternal factors in the production of congenital malformations.

A pilot study has shown that maternal factors may play a role in the causation of some malformations. An extensive study utilizing all available malformation data through age 7 years is being planned and will be designed when the 7-year data become available.

X. Study of the effects of major and minor malformations on outcome at 7 years.

This part will investigate the effects of major and minor malformations on neurological outcome, psychological test performance and speech, language and hearing performance. The study will be designed when the 7-year data become available.

XI. Correlation of minor chromosomal variants with congenital malformations.

This part will be carried out as a contract operation with Dr. Herbert Lubs, University of Colorado, Denver, as principal investigator. The study will utilize data which have been developed by five Collaborating Perinatal Project institutions which are currently participating in the Denver-based chromosomal study of minor variants.

The administrative mechanism for contract award has been initiated.

Keyword descriptors: Congenital malformations, singletons, twins, epidemiology, genetics, environmental factors, blood groups, chromosomal aberrations, growth and development.

Honors and Awards: None

Publications:

Willerman, L., Naylor, A.F. and Myrianthopoulos, N.C.: Intellectual development of children from interracial matings: performance in infancy and at 4 years. Behav. Genet. 4:83-90, 1974.

Jones, K.L., Smith, D.W., Streissguth, A.P. and Myrianthopoulos, N.C.: Outcome in offspring of chronic alcoholic women. Lancet 1:1076-1078, 1974.

Myrianthopoulos, N.C. and Chung, C.S.: Congenital malformations in singletons: epidemiologic survey. Birth Defects 10:1-58, 1974.

Chung, C.S. and Myrianthopoulos, N.C.: Analysis of epidemiologic factors in congenital malformations. Birth Defects, in press.

Myrianthopoulos, N.C. and Burdé, B.: A case of conjoined twins. Acta Genet. Med. Gemellol., in press.

Myrianthopoulos, N.C.: Congenital malformations in twins. Acta Genet. Med. Gemellol., in press.

Project No. Z01 NS 02110 - 02 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Delayed Motor Development at One Year: Antecedents and Intellectual and Neurological Outcomes

Previous Project Number: NDS (CF)-74 PR/PSC 2110

Principal Investigators: Sarah H. Broman, Ph.D., PRB,NINCDS
Karin B. Nelson, M.D., PRB,NINCDS

Cooperating Unit: None

Man Years:

Total	.02
Professional	.02
Other	.00

Project Description:

Objectives: A diagnosis of delayed motor development at one year made a significant independent contribution to IQ variance at age four, and was the best discriminator between retarded and normal children at that age. The purpose of this study is to define this condition more precisely in terms of specific development levels, and to examine some of its antecedents and longer-range outcomes at age seven. Factors to be investigated include maturity at birth, physical growth, presence of neurological and other medical diagnoses at one and at seven years, socioeconomic status and education of the parents, and IQ at age seven.

Proposed Course: Analyses of these data are continuing.

Keyword Descriptors: Motor development, IQ, mental retardation

Honors and Awards; None

Publications: None

Project No. Z01 NS 02112-02 PR
1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Neonatal Hyperbilirubinemia

Previous Project Number: NDS (CF)-74 PR/OC 2112

Principal Investigators: Joseph S. Drage, M.D., Chief, Perinatal Research Branch, C&FR, NINCDS
Janet B. Hardy, M.D., Associate Professor of Pediatrics, The Johns Hopkins University
Esther Jackson, Head, Section on Applied Statistics, Office of Biometry, C&FR, NINCDS

Other Investigators: None

Cooperating Units: The Johns Hopkins University; Perinatal Research Branch, C&FR, NINCDS: Office of Biometry, C&FR, NINCDS

Man Years:

Total:	.1
Professional:	.05
Other:	.05

Project Description:

Objectives: The neonatal hyperbilirubinemia study has been designed to assess the relationship of intermediate levels of serum bilirubin on the subsequent neurological and mental development of Collaborative Perinatal Project children. There has been increasing concern that neonatal serum bilirubin levels between 10-20 mg.% may be damaging to the central nervous system; not in the classical sense of "kernicterus" associated with levels above 20 mg.%, but rather damaging in more subtle yet clinically significant ways. Neonates are to be studied in five birthweight-gestational age categories, by three socioeconomic classes, for a variety of outcome measures, including mental and motor assessments at 8 months of age, and a spectrum of neurological findings at age one year, which will include motor performance, reflexes, tone, abnormal movements, eye findings, and the over-all neurological classification of normal, suspect, or abnormal. The analysis of phase I has been completed, tables and charts completed, and a draft manuscript is underway. Plans call for submission of a paper for publication near the end of the current fiscal year.

Project Descriptors: neonatal hyperbilirubinemia, bilirubin, neurological and mental development, perinatal, "kernicterus", birthweight-gestational, socio-economic, mental and motor, neurological

Honors and Awards: None

Publications: None

Project No. Z01 NS 02169 - 01 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Long Term, Differential Effects of Obstetrical Medication on Infants

Previous Project Number: None

Principal Investigators: Sarah H. Broman, Ph.D., PRB, NINCDS
Yvonne Brackbill, Ph.D., Georgetown University Hospital, Washington, D.C.

Cooperating Units: Georgetown University Hospital

Man Years:

Total	.15
Professional	.14
Other	.01

Project Description:

Objectives: The two-fold purpose of this study is to determine how long-lasting are the effects of obstetrical medication on infant psychophysiology, and to determine how long they continue to affect functions differentially. It has been reported that both anesthetics and analgesics depress the quality of infant test performance and that the drug produced dysfunction is not short-lived. Items from examinations at 4, 8, and 12 months of age that reflect CNS integrity will be analysed.

Proposed Course: The initial phase of the study is underway beginning with an analysis of the frequency with which specific pharmacological agents were used during labor and delivery in a cohort of normal births in the CPP, preliminary to analyzing relationships between obstetrical medication and infant outcome variables.

Keyword Descriptors: Obstetrical medication, infant outcome

Honors and Awards: None

Publications: None

Project No. Z01 NS 02170 - 01 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Offspring of Schizophrenics: Developmental Factors Related to Intelligence

Previous Project Number: None

Principal Investigators: Ronald O. Rieder, M.D., LP, DCBR, NIMH
Sarah H. Broman, Ph.D., PRB, NINCDS

Cooperating Units: National Institute of Mental Health

Man Years:

Total:	.3
Professional:	.2
Other	.1

Project Description:

Objectives: The objectives of this study are: 1) to assess the hypothesis that offspring of schizophrenics are more susceptible to perinatal complications than are the offspring of normals; and 2) to determine if the offspring of schizophrenics show a consistent pattern of abnormal development over time, extending from the neonatal period through childhood. Correlations between perinatal and early childhood events and later tests of intelligence in the offspring of schizophrenics group are being compared with those in a large normal group.

Preposed Course: Analyses of these data are continuing.

Keyword Descriptors: Offspring of schizophrenics, perinatal complications, intelligence

Honors and Awards: None

Publications:

Rieder, R. O., Rosenthal, D., Wender, P., and Blumenthal, H.: The Offspring of Schizophrenics: Fetal and Neonatal Deaths. *Arch. Gen. Psychiatry* 32: 200-211, 1975.

Project No. Z01 NS 02171 - 01 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Compendium of Heritable Disorders of the Nervous System

Previous Project Number: None

Principal Investigator: Dr. N.C. Myrianthopoulos, PRB, NINCDS

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.10
Professional:	0.05
Other	0.05

Project Description:

Objectives: To prepare a comprehensive list of all known heritable disorders of the nervous system, including disorders and malformation syndromes which, though not primarily neurological, have neurological involvement.

Methods employed: Sources for the compendium are published reports in the past and current literature containing convincing evidence of familial occurrence.

Current status: The available reports to date, yielded over 900 disorders. These have been classified into 23 nosological categories, including malformations, spinal atrophies, ataxias, demyelinating disorders, epilepsies, metabolic disorders, neuromuscular disorders, neoplasms and vascular disorders, mental retardation, syndromes, and others. A first draft of the list has been produced and is now being critically reviewed.

Keyword descriptors: Neurological disorders, CNS malformations, genetics, familial occurrence.

Honors and Awards: None

Publications: None

Project No. Z01 NS 02172 - 01 PR
1. Perinatal Research Branch
2. Professional Staff Consultants Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Children with Moderate or Severe Cerebral Palsy and Severe Mental Retardation

Previous Project Number: None

Principal Investigators: Karin B. Nelson, M.D., PRB, NINCDS
Sarah H. Broman, Ph.D., PRB, NINCDS

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.3
Professional:	0.2
Other:	0.1

Project Description:

Objectives: The gravity of disability of children with both cerebral palsy and severe mental retardation is so great that none is likely to become an independent citizen. Many are institutionalized, at a cost of approximately $7000 per year per child. Each child so handicapped represents both a human tragedy and a very substantial societal burden.

In addition to the social and medical importance of this group, its study was justified by the likelihood that in such a severely and multiply damaged cohort, the relationship between biological antecedents and outcome might be more readily analyzable than in less severely compromised samples.

Methodology: From a population of 45,300 children, 90 children were found who had moderate or severe degrees of cerebral palsy and an IQ below 50 at 7 years, or met these criteria at one year and died before seven. Comparisons were made with a control population of more than 30,000 children unselected for motor or mental characteristics, with respect to 53 variables relating to maternal and family characteristics, obstetrical factors, and neonatal findings.

Major Findings: The double disability of CP and severe mental retardation is approximately as common as Down's syndrome (mongolism). Of the ninety children with both CP and severe mental retardation, half were instituionalized by seven years. Seventy per cent survived at least to their seventh birthdays. Thirteen (14%) acquired their grave disabilities after the first month of life. Of factors significantly associated with serious mental and motor handicaps among the 77 congenital cases, the most striking relationships were found among neonatal characteristics. In particular, four groups of factors appeared especially important: 1) small size at birth (low weight, length, head circumference), 2) difficulty initiating and maintaining independent respiration, 3) low hematocrit and hemoglobin, and 4) neonatal seizures. Some aspects of the interactions of these factors has been investigated, and further analysis is underway.

Significance to Biomedical Research: It is a major concern of the Collaborative Perinatal Project to identify antecedents to childhood neurological disability, to suggest where preventive efforts may be directed with best effect. The present study suggests that, for the extremely disabled, neonatal abnormalities and certain observations during labor are much more strongly related to bad outcome than earlier predictors involving maternal and family characteristics, and that interventions focussing upon labor and neonatal periods may be most productive in attempting to prevent such very unfavorable outcomes.

Proposed Course: Multivariate analysis is planned to examine in greater detail the interaction of the variables found to be most related to bad outcome in this group. We will also examine further the relationship between the double disability under investigation and the larger groups of the cerebral palsied and mentally retarded who do not meet the criteria for this cohort.

Keyword Descriptors: Cerebral palsy, neonatal period, mental retardation

Honors and Awards: None

Publications: None

Project No. Z01 NS 01144-13 OD
1. Office of the Director
2. Office of Planning & Evaluation
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: An Instrument for the Conduct of a Retrospective Study of Seizures, Cerebral Palsy, Mental Retardation and Other Neurological and Sensory Disorders of Infancy and Childhood.

Previous Serial Number: NDS-63 OD/OPE 1144

Principal Investigators: Z. A. Shakhashiri, M.D., OPE, NINCDS
Leonard V. Phelps, Clearwater, Florida
Glen S. Bartlett, M.D., Case Western Reserve Univ., Cleveland, Ohio

Other Investigators: Lenore Bajda, M.D., PRB, NINCDS
John R. Day, M.D., Chevy Chase, Md.
Blanche L. Vincent, S.N.O., Greensboro, N.C.
Zula C. Meekham, B.S.N. PRB, NINCDS
Rose R. Tortorella, PRB, NINCDS

Cooperating Units: Georgetown University Hospital, Retarded Children's Clinic, Selected Maternity Hospitals and Physicians in Metropolitan Washington

Man Years:

Total:	.30
Professional:	.25
Other:	.05

Project Description:

Objectives: Design an instrument for the conduct of a retrospective study of seizures, cerebral palsy, mental retardation and other neurological and sensory disorders of infancy and childhood in order to test certain basic and important hypotheses concerning the occurrence of neurological damage.

Methods Employed: Recognized damaged outcomes of pregnancy, especially mental retardation, have been studied and related to defined perinatal or postnatal events which are involved in the biological or psycho-sociological mechanism underlying the following hypotheses: (1) anoxia, (2) toxic influences on the brain, (3) metabolic influences, (4) trauma to the head, (5) infection of the brain, (6) dehydration of the child, (7) genetic or familial patterns, (8) socio-economic status, (9) prematurity, and (10) nutrition.

Current Status: Upon review the manuscript was criticized on the basis of possible bias inherent in incomplete data as presented. Since the data base is available on tape, another extended analysis is under way, comparing as well, the emerging findings from this retrospective study with those from the prospective COLR study when subjected to the same instrument. The validation of findings from the retrospective study would thus be possible.

Keyword Descriptors: Retrospective study, mental retardation, infancy, childhood, perinatal events, postnatal events, biological, psycho-sociological, anoxia, toxic, metabolic, trauma to the head, infection, dehydration, familial, socio-economic, prematurity

Honors and Awards: None

Publications: None

Project No. Z01 NS 01146-13 OD
1. Office of the Director
2. Office of Planning & Evaluation
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Public Health Implications Study of Perinatal Mortality in the Collaborative Study and in the Collaborative Study Cities.

Previous Serial Number: NDS-63 OD/OPE 1146

Principal Investigators: Z. A. Shakhashiri, M.D., OPE, NINCDS
Leonard V. Phelps, Clearwater, Florida
Glen S. Bartlett, M.D., Case Western Reserve Univ.
Cleveland, OHIO

Other Investigators: None

Cooperating Units: The Census Bureau and the National Center for Health Statistics cooperated in the furnishing of necessary statistical information for the United States and cities. The respective state or city health departments provided natality and mortality data for the Project.

Man Years:

Total:	0.05
Professional:	0.05
Other:	0.00

Project Description:

Objectives: To evaluate fetal and infant mortality of the Collaborative Study population and of the cities from which that population is drawn, with the aim of comparing the two populations, city by city, and institution by institution, on mortality characteristics.

Methods Employed: In addition to the data previously available from the National Center for Health Statistics for perinatal events, detailed data on natality and perinatal mortality were sought for the study cities from either the state or city health departments, whichever had jurisdiction for these records. The data include figures for livebirths, stillbirths, and deaths under 24 hours, 1 day to 7 days, 8 days to 28 days and 1 month to 12 months, evaluated by birth weight, length of gestation, race and sex, and plurality for the years 1959 through 1966. Corresponding data were compiled by institution for the PRB study population. The state or city health departments furnished either completed tabulations or raw data to be tabulated.

Considerable effort has been expended to create a usable data file of the external data being obtained in connection with this study. The aim is to provide a file with more general utility than the limited scope of this study. When such a file is created, the information necessary to make use of the file will be made available to interested persons.

Current Status: There has been an unavoidable delay in the preparation of the manuscript describing the comparative trends of fetal, neonatal and infant mortalities in the COLR population and in the populations of the cities where COLR institutions are located.

Keyword Descriptors: Public health implications, perinatal mortality, infant mortality, livebirths, birth weight, gestation length, race, sex, plurality.

Honors and Awards: None

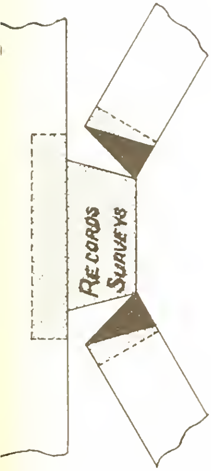
Publications: None

HOW TO USE
THESE SEPARATORS

Use one page for each separation.

Select appropriate tab, add further identification if desired, and cover it with scotch tape.

Cut off and discard all tabs except the one covered by tape.



TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

ANNUAL REPORT

July 1, [illegible] through June 30, [illegible]

SPECIAL PROGRAMS BRANCH
Collaborative and Field Research

National Institute of Neurological and Communicative
Disorders and Stroke
National Institutes of Health

The Special Programs Branch continued in operation in Fiscal Year [illegible] with Mr. Alfred Weissberg as Acting Chief and with the same responsibilities, namely:

1. The development, conduct, and direction of programs for scientific and technical information exchange relevant to the neurologic and sensory sciences on a national and international basis.

2. Coordination of collaborative research and development studies with Institute goals and objectives carried out in foreign laboratories with U.S. Government support under Public Law 480 (PL 480).

3. Development and conduct of national and international directed research programs of relevance to the Institute's goals and in response to Institute program needs and priorities as identified by the Director, NINCDS and the Associate Director, CFR, NINCDS.

A major activity during the year was the in depth review of each information center. Site teams reviewed the activities at the Brain Information Service, UCLA, and The Information Center for Hearing, Speech, and Disorders of Human Communication, JHU. The Clinical Neurology Information Center, University of Nebraska, was reviewed by its national advisory committee. The results of the reviews were communicated to the Institute's Science Information Program Advisory Committee, which provides recommendation to the Institute regarding the operation of each center.

In all three instances, the Advisory Committee's recommendations [illegible] with those of the site review teams. The National Advisory Neurology Council also discussed the proposed restructuring of the information centers and supported the Science Information Program Advisory Committee's recommendations.

The Institute, in following the results of this review process intends to terminate the operation of the Center at The Johns Hopkins University at the close of the contract year on October 31, 1975; to reduce the funding of the Brain Information Service, UCLA, to a level consistent with the advisory recommendations and based on the delivery of a limited number of specified products; and to maintain the operation of the Clinical Neurology Information Center, University of Nebraska, at its current level of activity and funding.

By a joint effort of BIS and NINCDS, the BIS is to assemble a nationally representative group of advisors to BIS to monitor and evaluate its products.

Support was continued for the preparation by the Mayo Clinic of cerebrovascular disease abstracts for publication in the journal STROKE. Also, the Bulletin on Parkinson's Disease and Related Disorders continued to be distributed through the National Technical Information Service. Office of Management and Budget clearance for this publication was granted to March 31, 1978.

The publication "Parkinson's Disease and Related Disorders" is a monthly bibliography from the NLM data base that is distributed by the National Technical Information Service (NTIS) at a cost of $20 per year to subscribers. The NINCDS, which formerly distributed the publication at no charge, saves approximately $4,000 per year by this arrangement. The publication was sent to a user group of approximately 1000 before being placed on a subscription basis. Its circulation now is over 200 and growing.

Currently, all of the products from the information centers are on a fee for service basis. The income from the fees covers the cost of printing and distribution of the products, but is not adequate to allow any center to be self sufficient.

The Institute, in restructuring the activities of the Information Centers expects to reduce its annual rate of expenditure from $825,000 to $500,000. To achieve this reduction, the budget of the Brain Information Service at UCLA is to be reduced and the Communicative Disorders Center at JHU is to be discontinued. The budget for the Clinical Neurology Information Center and the Cerebrovascular Disease Abstracts are expected to remain the same.

Negotiations with the National Library of Medicine were initiated to establish the Epilepsy Abstracts Data Base as an adjunct to the NLM MEDLINE on-line retrieval system to be available to epilepsy centers and neurologists nationwide.

Special Report
Foreign Currency Credit Award Programs
(PL 480)
Fiscal Year 1975

The foreign currency credit award or PL 480 Program is a program of research supported by United States owned foreign currencies which are not convertible to dollars. It is authorized by the Agricultural Trade Development Systems Act, 1954, Public Law 480, subsection 104K, which permits the use of foreign currencies, "to conduct and support scientific activities overseas, including programs and projects of scientific cooperation between the United States and other countries such as coordinated research against diseases common to all mankind, that are unique to individual regions of the globe, and promote and support programs of medical scientific research."

In Fiscal Year 1975, NINCDS had 29 ongoing projects. There was one project in Egypt, nine in India, one in Israel, six in Poland, and twelve in Yugoslavia. Funds are available in Pakistan and Tunisia, but we have no projects in these countries. The scientific content of the PL 480 research is complementary to other NINCDS research and covers both clinical and laboratory studies.

Each of the PL 480 projects must have a U.S. scientist to serve as a collaborating "Project Officer" who works with the foreign scientist in varying degrees. The role of the U.S. project officer varies from that of an active collaborator participating in the research to that of an informed sponsor. However, the project officer must each year review the expenditures and the progress report and advise the Institute whether the progress has been satisfactory. Of the 29 projects, ten have project officers who are in the NINCDS. The other 19 project officers are in universities throughout the country.

It has always been difficult to find scientists who are capable of doing research even in countries that are fairly well developed. The former NIH international fellows who have trained in this country and are working in the areas of interest to NINCDS in their native lands have been contacted to see if they can develop suitable projects. This has met with some success, and many of our developing projects are from former fellowship holders.

In Yugoslavia the involvement of other U.S. agencies in the PL 480 program has resulted in a gross overplanning for the use of available funds. A joint U.S.-Yugoslavian committee has worked out an extension of the program with contributions from both the United States and Yugoslavian sides. Available funds for the Yugoslavian activities have been used up! New projects must be done on the basis of U.S. dollars matched by Yugoslav dinars. No great interest in doing this has been shown.

There is a large amount of money in Egypt available for PL 480 research projects, but there is difficulty in finding qualified people. The number one priority health problem is schistosomiasis, followed by infectious diseases (including meningitis). Egyptian researchers are interested in the neurological diseases, particularly accidents and injuries to the CNS. However, the facilities are very poor and modern equipment in the laboratories is scarce. Clinicians and scientists who have trained outside of the country are well trained and capable. They are limited by the lack of facilities and financial support. Also, there is very little infrastructure of technicians or facilities for repair and maintenance of equipment. This is a reflection of the fact that there is only about 40% literacy in the country.

In Poland, the funds available for use have been sharply curtailed. The amount available for projects on neurological and psychiatric disorders effectively limits the number of possible projects to a very few. In light of this, we are forced to examine carefully the proposals and to assign priority rankings for the Institute and to collaborate with NIMH in an overall ranking for both areas.

The PL 480 program in Israel is almost totally phased out because all funds have been used. The current projects are as follows:

Egypt	03-006-1	Venoms of Poisonous Snakes
India	01-011-1	Nutritional Disorders of Nervous Systems
	01-015-1	Neural Mechanisms in Regulation of Feeding Behavior
	01-016-1	Neurologic Investigations of Cerebrovascular Disorders
	01-024-1	Collaborative Neurologic Studies
	01-034-1	Neurotoxins from Plan Materials
	01-040-1	Polypeptide Constituents of Snake Venoms
	01-041-1	Effect of Protein Malnutrition on Brain Metabolism
	01-109-1	Uptake of Drugs
Israel	06-015-1	Synthesis of Sphingolipids
Poland	05-002-1	Characteristics of Muscle Disease
	05-004-1	Biology and Pathology of the Neuron and the Glia
	05-013-1	Biology and Histochemistry of Gliomas
	05-027-1	Biochemistry of Myelination and Demyelination
	05-028-1	Anoxemic Damage of the Immature Nervous System
	05-035-1	Brain Fine Structure in Rabbits with Hereditary Tremor

Yugoslavia	02-003-1	Neural Correlates of Behavior
	02-006-1	Histogenesis of the Forebrain in the Human Embryo
	02-008-1	Function of Cholinesterases
	02-015-1	Metabolism. Release and Uptake of 5-Hydroxytryptamine
	02-023-1	Vestibular Influences on Cerebral Activity
	02-020-1	Function of Biosynthesis of Ribosomes
	02-025-1	Neuropathology of Close Brain Injury
	02-023-1	Sialidase Action on Complex Molecular Aggregates
	02-041-1	Synaptic Transmission in Brain
	02-042-1	Brain Survival in Anoxia and Hypothermia
	02-090-1	Gene Transcription and Gene Expression in Sea Urchin Embryos

Project 01-015-1 is concerned with the central and autonomic control system modulating alimentary receptor activities and regulation of feeding behavior. Project 02-003-1 is a large program to understand the function of the nervous system by studying physiological and biochemical correlates of behavior. In conjunction with this study is the development of a major research source, the International Brain Research Laboratory in Yugoslavia.

Five projects are basically neurochemical or metabolism chemistry studies, but they are generally unrelated. Project 02-042-1 is a study of the tolerance limiting role of metabolic events occurring in the brain at conditions of anoxia induced at different levels of hypothermia down to zero degrees Centigrade. Project 05-027-1 is a study of the role of neuroglia cells in the myelination and demyelination with emphasis on the enzyme chemistry of the processes. This relates to human demyelinating diseases such as multiple sclerosis. Project 05-004-1 is somewhat similar and is a study of the glia of the nervous system and their function in diseases of the brain. Particular attention will be given here to the intracellular oxidation reduction enzymes. Project 02-032-1 deals with the maintenance of the surface characteristics of membrane systems and the enzyme actions required to maintain these membrane systems . The control and regulation of protein synthesis in biological systems is the focus of Project 02-020-1.

There is one neuropathological study, Project 05-035-1, which is a study of the fine structure of the brains of rabbits with hereditary tremor. This tremor is similar to that seen in Parkinson's disease and this study may have some bearing on that disease.

There are six studies which relate to the neurological diseases of children. One of these, Project 06-015-1, is purely biochemical in nature; to synthesize the sphingolipids of the brain. This has been important because these substances have been used to elucidate the enzymatic defects of such diseases as Tay-Sachs disease. Another study, Project 01-024-1, is both biochemical and clinical and deals with the enzymatic processes involved in the sulfation and desulfation of glycosaminoglycans and cerebroside sulfate. These biochemical mechanisms have been studies in specific metabolic disorders of children. Using material from these patients the scientists working on this project have identified specific enzyme defects. There are two neuropathological studies relating to childhood. Project 05-006-1 is a study of the histogenesis of the forebrain in the human embryo, in which material from products of abortions is used. The other, Project 05-028-1, is a neuropathological study of the anoxia damage of the immature nervous system in the brains of newborns and infants who succumb to early anoxia. There are two studies that are related to nutrition of the brain. Project 01-041-1 studies the effect of protein malnutrition on the brain, metabolism, and development. Project 01-011-1 is a general study of the nutritional disorders of the nervous system particularly those defects associated with malnutrition, the malabsorption syndrome, and peripheral neuropathies of uncertain etiology.

There are two projects relating to trauma of the nervous system. Project 02-025-1 is a neuropathological study of closed brain injuries to determine the topographic distribution and histopathology of closed head injuries. Project 01-009-1 is a study of the role of arterial venous malformations of the spinal cord which cause paraplegia and other myopathies of unspecified origin.

Project 01-016-1 is a study of clinical angiographic and anatomic aspects of cerebrovascular disease in India. Project 05-002-1 is an extensive multiple disciplinary program for the study of muscle disease in Poland. Project 05-013-1 is a study of the biology and histochemistry of gliomas using tissue culture and immunochemical methods. Project 02-023-1 on the vestibular inputs contribute to the spread of cerebral electric rhythms and to correlate these with clinical problems. Project 02-090-1 is a new study on gene transcription and gene expression in Sea Urchin Embryos.

There are projects concerned with naturally occurring neurotoxins. Of these, Project 03-006-1 is concerned with obtaining material of the poisonous animals such as snakes from the Middle East, North Africa, and as far South as the Union of South Africa. Biochemical and physiological studies will be carried out on the purified toxins which will also be developed. Project 01-040-1 is a similar study being carried out in India, studying the poisonous snakes of India. The third Project, 01-034-1, is on neurotoxins from plant materials, and is focusing on toxicity of B-disminopropionic acid (ODPA) which is extracted from the seeds of Lathyrus Sativus in India. This will be studied in connection with the development of human lathyrism.

There are five projects concerned with synaptic transmitters. Project 02-027-1 is to purify and characterize acetylcholinesterase and to determine its molecular weight and other chemical characteristics. Project 02-008-1 is to determine the role of the acetylcholinesterases in the acetylcholine system transmission conduction of excitation. There are two others which are somewhat related in the study of the metabolism release of 5-hydroxytryptamine and its function as a transmitter in the brain, Projects 02-015-1 and 02-041-1. These four are in Yugoslavia. The fifth project, in India, Project 01-109-1, is a study of factors which alter the uptake and retention in various tissues of catecholamines and drugs which affect their action. The drugs studied are primarily the catecholamines, catecholamine depletors, and other drugs which either mimic the effect of catecholamine or block it.

Contract Narrative
Special Programs Branch, C&FR, NINCDS
Fiscal Year 1974

The Brain Information Service at UCLA (NIH-NINDS-70-2063)

Title: Operation of Specialized Information Center on Nonclinical Neurosciences

Contractor's Project Director: Michael H. Chase, Ph.D.

Current Annual Level: $412,000

Objectives: To operate a specialized information center for the nonclinical neurosciences to serve as a national focal point for the identification, collection, storage, retrieval, analysis, repackaging, and dissemination of information. The major thrust of this information center shall be information analysis products and services. The center will identify and locate all information items and services pertaining to its subject area. The center will use the identified and stored information to make available comprehensive information service primarily to the scientists supported by NINCDS, secondarily to the remainder of the biomedical community throughout the country, and, when possible, internationally. These services shall include: (a) current awareness services; (b) bibliographic services; (c) translation of foreign language articles when essential; (d) answering scientific questions relating to the subject area; (e) promotion and preparation of reviews and other special articles for the general as well as specialized segments of the biomedical community; organizing and supporting workshops on specialized topics in the brain sciences and publishing their proceedings where necessary; (f) furnish information about ongoing research; (g) preparation of a publication series in the brain sciences.

Major Accomplishments: The Brain Information Service reached a level where relevant scientific information was sent yearly to approximately 10,000 scientists. This was prior to fee charges for all products. This past year showed changes due to these charges. The major current alerting bulletins produced are:

1. Sleep Bulletin including Sleep Reviews is a monthly bulletin with 750 subscribers. These bulletins are so useful that the Association for the Psychophysiological Study of Sleep has requested that all members have copies of the bulletin and have made subscription to them a requisite for membership in the association. The subscription fee is $22 per year.

2. Biogenic Amines and Neurotransmitters in the Nervous Systems: an annotated bibliography which appears semi-monthly and contains

contains several one-page reviews or abstracts in depth. Distributed to 1,000 at a fee of $23 per year.

3. Neuroendocrine Control Mechanism: Hypothalmic-Pituitary-Gonadal System - an annotated semi-monthly with several one page reviews or abstracts in depth. Distributed to 650 subscribers at a fee of $24 per year.

4. Memory and Learning - Animal Studies: a monthly listing of published studies. Distributed to 430 subscribers for a fee of $19 per year.

5. Memo of Current Books in the Brain Sciences: comes out monthly and is annotated. Distributed to 410 subscribers for a fee of $18 per year.

The Index to Current (EEG) Literature is prepared by the BIS as a quarterly supplement to the journal Electroencephalography and Clinical Neurophysiology. The cost of printing and distribution is borne by the EEG Society.

The Sleep Research series, Volume 3, 1974, contains expanded abstracts of the year's Association for the Psychophysiological Study of Sleep meeting, current claims of research findings and all pertinent bibliographic information for the year.

The Conference Report Series has been continued. These reports have been in great demand with as many as 3500 requests for a single report. Significant new reports produced this period are:

1. Society for Neuroscience 4th Annual Meeting

2. 8th Annual Winter Conference on Brain and Behavior

Other products completed are:

1. Translation of a Soviet Research Report on the Auditory System by Altman

2. Three Volume Review on Synapses by Bubis and Sandbank

3. A Cumulative Bibliographile of the Alerting bulletins and one on EEG recording in the CNS.

4. "Operant Control of Brain Activity", Volume 2, Perspectives in the Brain Sciences.

5. Reference Bibliographies on:

   a. Neural Mechanisms of Pain
   b. Psychosurgery (in man)
   c. Enzymes Involved in Neurotransmitter Synthesis and Metabolism

6. Recurrent Bibliographies (quarterly):

   a. Contingent Negative Variation
   b. Cerebral Evoked Potentials
   c. Proteins in the Brain
   d. Inborn Errors of Metabolism: Physiology and Chemistry
   e. Neuroimmunology

Significance to Biomedical Research: The Brain Information Service performs an essential service to the biomedical community by distilling and repackaging all new information items within its area of responsibility. An indication that this program is becoming central to the advancement of research in the basic brain sciences is the formal relationship being developed with the BIS by the Society for Neurosciences in which the Society depends more and more on the BIS as a resource to the neuroscience community. The BIS mailing list numbers more than 16,000 scientists.

Proposed Course of the Contract: This program is under the surveillance of the NINCDS project officer and its performance is reviewed periodically. Continued funding for this program will be greatly reduced.

Contract Narrative
Special Programs Branch, C&FR, NINCDS
Fiscal Year 1975

Clinical Neurology Information Center at the University of Nebraska
(NIH-NINDS-72-2300)

Title: The Operation of a Clinical Neurology Information Center

Contractor's Project Director: Walter J. Friedlander, M.D.

Current Annual Level: $134,000

Objectives: To operate a specialized Information Center on Clinical Neurology. This Center will be an international focal point for information relating to those diseases of interest to NINCDS, especially information relating to diagnosis, treatment and prevention of diseases of the brain and central nervous system. The Center is producing reviews of various clinical problems of interest to the Government, bringing together the relevant clinical knowledge as it applies to the problem. These reviews may focus on an entire disease problem as a whole, or on any distinct part of a disease entity. The Center will identify sources of information relevant to clinical neurological problems, including indexing services, abstracting services, periodical journals, books, monographs, etc.

Major Accomplishments: In its third year of operation, the Center is obtaining critical reviews from leading clinical specialists. The manuscripts completed are the following:

1. Developmental Dyslexia: Neurological Aspects
2. Minor Hemisphere Syndrome
3. Evaluation of Speech Therapy in Acquired Aphasia
4. Clinical Evaluation of Neuropsychological Tests

These were published in a single volume by Raven Press, Volume 7 of Advances in Neurology.

The Center's most innovative product and one that has been received enthusiastically by the approximately 1000 scientists who receive it is the Concise Clinical Neurology Review. This publication emphasized a one sentence (terse) abstract of each paper in a cluster of terse statements on a single topic. A number of these topics are covered in a single issue of the bulletin. The bibliographic citations are referenced by a number and appear together in a second part of the bulletin. Approximately 300 papers are selected for inclusion each month, based on a review of 818 serials. This publication seems to fulfill a need not otherwise met in neurology. It is produced once every two weeks and has a subscription rate of $15 per year.

Proposed Course of the Contract: This program is under the surveillance of an NINCDS project officer and its performance is under continued review.

Contract Narrative
Special Programs Branch, C&FR, NINCDS
Fiscal Year 1975

Information Center on Hearing, Speech, and Disorders of Human Communication at The Johns Hopkins University (NIH-NINDS-71-2281)

Title: Operation of an Information Center on Human Communications

Contractor's Project Director: John E. Bordley, M.D.

Current Annual Level: $260,000.00

Objectives: To operate a specialized information center on speech, hearing and human communication to serve as a national focal point for the identification, collection, storage, retrieval, analysis, repackaging, and dissemination of information. The major thrust of this information center shall be information analysis products and services. The center will identify and locate all information items and services pertaining to its subject area. In addition, the center will use the identified and stored information to provide comprehensive information service to the biomedical community throughout the country and, when possible, internationally. These services shall include: (a) current awareness services; (b) bibliographic services; (c) translation of foreign language articles when essential; (d) answering scientific questions relating to subject area; (e) promotion and preparation of reviews and other special articles for the general as well as specialized segments of the biomedical community; (f) furnish information about ongoing research and (g) develop new methods of information analysis and dissemination.

Major Accomplishments: Current Citations on Communications Disorders is the major current awareness product of the Center, having circulation of 1,200. This bulletin is divided and distributed in two parts: (1) Hearing and Balance, and (2) Language, Speech, and Voice. These bulletins provide up-to-date coverage of articles, editorials, chapters of books, small monographs unpublished papers, reports, etc. in the field of the communicative sciences. The audience includes scientists, clinicians, educators, administrators and students.

Biblioprofiles are comprehensive bibliographies on selected subjects that include a short state-of-the-art digest by a scientist expert in the subject area. These products are available for a nominal charge that covers the cost of reproduction and mailing. The following is a list of Biblioprofiles that have been produced this year.

Completed and/or Printed:

No. 10. Acquired Aphasia
No. 11. Language Development in the Normal Child

In preparation:

Language and Speech Education of the Deaf

Prepared Bibliographies are produced periodically and are comprehensive bibliographies on selected topics. These had been available free, but are now available for a nominal charge that covers reproduction and mailing. Those that have been produced this year are:

- Tympanosclerosis
- Serous Otitis Media in Children
- Speech Problems of the Deaf and Data About Normal Speech
- Ear Wax and Hearing Impairment
- Attitudes Toward Child With a Speech Problem
- All About Hearing Aids (for a Conference)
- Impedance Audiometry With Mentally Retarded Child
- Non-medical Management of Oro-facial Pathology
- Cerebral Dominance and/or Cerebral Laterality as a Cause of Neurological Dysfunction
- Role of Public School Speech Clinician
- Statistics on Speech and Language Problems
- Congenital Hearing Problems

In Preparation:

- Acupuncture for Hearing Impairment
- Dichotic Listening and Perceptual Asymmetries
- Voice Identification by Eye, Ear and Machine
- Audiometric Technique
- Hearing Aids: Properties and Prescription
- Industrial Audiology
- Audiologic Habilitation
- Stuttering
- Language Disorders in Children
- Learning Disabled Children
- Speech and Language Habilitation of the Mentally Retarded
- Speech Habilitation With Clefts and Other Oro-facial Anomalies
- Speech and Language Habilitation of the Cerebral Palsied
- Childhood Articulation Disorders
- Rehabilitation of Aphasic Adults
- Geriatric Hearing Impairment
- Systems and Methods of Communication of Chimpanzees

Significance to Biomedical Research: The Information Center for Hearing, Speech and Disorders of Human Communication performs a valuable service to the biomedical community by distilling and repackaging all new information items covering the many disciplines in its area of responsibility. Special attention is given to the information needs of those whose programs are supported by NINCDS funds.

Proposed Course of the Contract: This program is monitored by the NINCDS project officer and its performance is reviewed periodically.

There will be no continued funding for this project.

Contract Narrative
Special Programs Branch, C&FR, NINCDS
Fiscal Year 1974

Cerebrovascular Disease Abstracting Service at the Mayo Foundation
(PH-43-NINDS-66-933)

Title: Cerebrovascular Disease Abstracting Service

Contractor's Project Director: Robert G. Siekert, M.D.

Current Annual Level: $25,000.00

Objectives: To provide a bibliographic alerting service of new literature on cerebrovascular disease by preparing abstracts of appropriate articles for publication in STROKE - A Journal of Cerebral Circulation.

Methods: The abstracts are from pertinent articles culled from a review of about 148 serial journals. Either the author's abstract is used or a new one is prepared if the author abstract is too long or otherwise unsuitable. They are then given to the editors of STROKE for incorporation in the journal.

Major Accomplishments: The journal STROKE carries in each of its bimonthly issues approximately 100 abstracts prepared under this contract.

Significance to Biomedical Research: For several years, these abstracts were offered to a small group of researchers on an informal basis. As more and more scientists heard about this service, the demand for the abstracts became so great that a way of general distribution was needed. The publication of these abstracts as a special section in the journal STROKE makes the service available to all those who would find it useful.

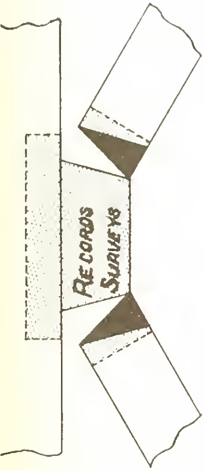
Proposed Course of the Contract: This program is under the surveillance of an NINCDS project officer and its performance is reviewed periodically.

HOW TO USE
THESE SEPARATORS

Use one page for each separation.

Select appropriate tab, add further identification if desired, and cover it with scotch tape.

Cut off and discard all tabs except the one covered by tape.



TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

ANNUAL REPORT
July 1, 1974, through June 1, 1975
Extramural Programs
National Institute of Neurological and Communicative Disorders and Stroke

TABLE OF CONTENTS

Subject — Page

Introduction 1

Fundamental Research in the Neurological Sciences
- Introduction 8
- The Synapse 9
- The Neurotoxins 12
- Genetic Determination in the Nervous System 15

Cerebrovascular Disorders 20

Central Nervous System Trauma
- Head Injury 25
- Spinal Cord Injury 28

Multiple Sclerosis and Other Demyelinating Disorders 33

Parkinson's Disease 41

Viruses and Neurological Disorders 52

Neuromuscular Diseases 65

Convulsive Disorders 68

Communicative Sciences
- Hearing and Deafness 75
- Cochlea-Inner Ear 79
- Hearing Disorders - Otitis Media 83
- Speech 84
- Language and Speech Disorders 87
- Taste and Olfaction 88
- Tactile Perception 90

Research Training (Special Programs Branch) 93

Annual Report
July 1, 1974, through June 30, 1975
Extramural Programs
National Institute of Neurological and Communicative Disorders and Stroke

Introduction

The greatest gift of man is the uniquely complex and mysterious organ called the brain. All that is specifically human derives from its intricate functioning with man's nervous system in the vital processes of life. The study of the billions of nerve cells comprising the brain and nervous system is a vast and difficult task. Nevertheless, scientists continue to labor to gain a greater understanding of these functions so intimately linked to the human life and creativity of each of us.

The Institute's mission is to promote and support research and research training in neurological and communicative disorders--disorders which afflict an estimated 20 percent of our population, from the youngest to the oldest, at an annual cost of some $20 billion. Because most of the disorders are chronic, lifetime afflictions, they have a tragic impact on the quality of life. They can mean loss of personal independence and often an end to schooling homemaking or wage-earning.

The research grant and research training programs of the National Institute of Neurological and Communicative Disorders and Stroke are focussed on the identification, stimulation and, support of essential research problems aimed at the improved diagnosis, treatment, and prevention of disorders of the nervous system, the neuromuscular apparatus, the ear, and human communication. They include disorders of the young (cerebral palsy, epilepsy, learning disabilities), of adulthood (head and spinal cord injury, multiple sclerosis, brain tumors), and of the aged (stroke, parkinsonism, otosclerosis). The administrative instruments used to accomplish these purposes include research projects, research program projects, clinical research centers, outpatient clinical research projects, specialized research centers, research career awards research career development awards, teacher-investigator awards, institutional research fellowship awards and individual research fellowship awards.

One of the most urgent needs is a safe and effective method to detect the danger of strokes before they occur. Strokes take about 200,000 lives in the United States each year and are exceeded only by head injury, heart disease and cancer. More effective forms of therapy are needed for epilepsy. No really new anticonvulsant drug has been available in the United States during the past ten years despite the fact that convulsions cannot be controlled completely in 25-30% of epilepsy patients. Only in recent years has there been full appreciation of the tremendous loss from accidents involving head and spinal cord injury. It is clear now that expert multidisciplinary approaches must be available within minutes after an accident in order to have the best possible prognosis. Parkinsonism afflicts about one out of every thousand people after the age of fifty. Although L-Dopa is one of the modern miracles of medicine, it has severe side effects and some 25% of the patients show little improvement. Therefore, L-Dopa probably will not be the ultimate drug of choice, but it has opened the door to a whole new area of study on the chemistry and function of the brain. Cerebral palsy is one of childhood's greatest cripplers. An estimated half-million people lack normal control of their muscles as a result of this brain-damaging disorder in infancy. These are some of the main areas in which research is being pushed within the limits of available resources.

Determined efforts to encourage research on communicative disorders, particularly deafness, have not been as successful as had been hoped. Since patients in this category are usually ambulatory, Outpatient Clinical Research Centers were established several years ago. In FY '69 after several years of work, the NANDS ad hoc Subcommittee on Human Communications and Its Disorders submitted a three-volume report with twenty-six specific recommendations and a detailed analysis of the present state of research and research training in this area. In FY '70 the Institute established a Communicative Disorders Program Project Review Committee which, in addition to reviewing applications, studied the needs in the field and identified laboratories where research on communicative disorders should be supported. The limited response to these efforts was compounded by the uncertainties and disruptions in the research training programs. Therefore, in FY '72 the Communicative Disorders Program Project Review Committee was combined with the Communicative Disorders Research Training Committee. The research grant and training areas are, of course, very similar. Many of the same investigators apply for both types of support and it seemed clear that one committee could handle the work load in the foreseeable future.

However, the Institute has continued to try to encourage research in this area. There is no lack of important problems. The detection and treatment of impaired hearing in infants alone holds great promise of untold savings in economic productivity and human suffering. The possible development of a useful auditory or cochlear prosthesis has recently attracted a great deal of attention. In essence consisting of a "hearing aid" with electrical connections directly to the auditory nerve, it may provide a semblance of speech or at least serve as a useful aid to many adults and elderly who have little or no residual hearing. The Institute has recently widely announced its interest in supporting research in this area.

Through the persistent efforts of the Director, the name of the Institute was changed to include the words "Communicative Disorders" to give greater visibility to this area. In the reorganization of the Extramural Programs, described elsewhere in this Report, Communicative Disorders is one of four main program areas. A senior scientist has been recruited to give leadership to the combined grant and contract activities in this area. Similar developments have been brought about in the Intramural Program with the establishment of the Laboratory of Neuro-Otolaryngology also under a newly recruited senior scientist.

It is clear that the Institute continues to do everything possible to stimulate interest in this area. It is equally clear that these efforts barely scratch the surface. Of all professional groups to which this Institute has a responsibility, this group is by far the largest, consisting of some

25,000 audiologists, speech pathologists, and otolaryngologists. With so many people, why so little research activity? The reasons are obvious. The training of audiologists and speech pathologists is strongly oriented toward public health problems leading to employment in public schools and community colleges and usually not going beyond the Masters level. Little or no research training is included. Otolaryngologists spend many years in medical and specialized clinical training. Even with some special financial assistance few can afford to take time out for research training. After they complete their clinical training, they are faced, even on medical school faculties, with terrific pressures for patient care since people with speech and hearing disorders probably constitute the largest single patient population in the U.S. The long range need obviously is more encouragement and support for research training. The uncertainties and disruptions in the research training program in recent years are, therefore, particularly regrettable.

The Extramural Program continues to utilize workshops and working conferences as one approach to an analysis of developing areas or areas with special problems. During the past several years, the use of psychosurgical methods for the treatment of uncontrollable violence and rage behavior generated a great deal of controversy. At the request of DHEW, NINCDS last year invited 48 distinguished leaders in basic and clinical research to join in the preparation of a report based on four workshops (1) neuroanatomical and neurophysiologic studies; (2) neurochemical, endocrine, pharmacological, and genetic studies; (3) behavioral studies; (4) clinical studies. A summary of the Report on the Biomedical Research Aspects of Brain and Aggressive Violent Behavior has been published in the Archives of Neurology, Vol. 30, January 1974. The full report was forwarded to DHEW.

A National Advisory Commission on Multiple Sclerosis was appointed in February, 1973, by the Secretary, DHEW, in accordance with an act of Congress (P.L. 92-563). The charge to the Commission was: "To determine the most effective means of finding the cause of and cures and treatments for multiple sclerosis." In February 1974, the Commission submitted its report, "Report and Recommendations of the National Advisory Commission on Multiple Sclerosis," containing a series of recommendations on funding research, on budget planning, and on the management of research on multiple sclerosis.

As part of the Senate Report on the FY '74 Appropriation for NINDS, the Senate Appropriations Labor - HEW Subcommittee specified that "......this would be an opportune time for an independent, unbiased study of the fundamentals of the chiropractic profession. Such studies should be high among the priorities of the NINDS ......" In order to provide the substantive base necessary for this evaluation, the NINCDS convened a "Workshop on the Research Status of Spinal Manipulative Therapy." The Workshop was held at the NIH on February 2-4, 1975. Its participants included 58 scientists and clinicians of national and international stature, including 16 Doctors of Chiropractic (D.C.), 24 Doctors of Medicine (M.D.), 7 Doctors of Osteopathic Medicine (D.O.), and 11 basic scientists (usually Ph.D.). Participants came from the United States and eight foreign countries. A preliminary report has been submitted. The Workshop Proceedings are to be published as part of the NINCDS Monograph Series and will be available from the Government Printing Office.

The ability to induce the functional regeneration of severed nerve fibers in the spinal cord and peripheral nerve tracts obviously would be of the utmost importance. Not only might it eliminate most of the horrible crippling effects of spinal cord injury, but it might be very useful in many other neurological disorders such as stroke, multiple sclerosis, and head injury. Regeneration of the goldfish spinal cord has been established. Soviet scientists have reported the successful regeneration of the rat spinal cord. As a result of these developments and the interest of the U.S. Congress in spinal cord injury, the Director, NINCDS, at the request of the NANCDS Council, established a Council Ad Hoc Subcommittee on Growth and Regeneration in the Central Nervous System in October 1973. Its mission was to evaluate the current status of research on regeneration in the central nervous system and to identify those areas where potentially significant advances might be expected. This task was accomplished through four workshops and six solicited manuscripts to provide relevant background information. With this dedicated participation by some 50 of the top experts in the field a full Report on the Current Status of Research on Growth and Regeneration in the Central Nervous System was submitted to the Council in March 1975. The Report includes 17 specific recommendations on High Priority Research Areas, Training, and Planning and Coordination.

The following Table shows the number of research grant applications considered by the Council at its June meetings in each of the past five years.

	June '71	June '72	June '73	June '74	June '75
Number	352	398	467	428	481

During the first three years of this period the number of applications increased approximately 30 percent. This is attributed primarily to the effectiveness of the research training programs of the Institute in the output of fully trained investigators. During the past two years the number of applications seem to have leveled off. It is uncertain to what extent this is due to recent disruptions in the research training program or to the limitations on available funds in recent years discouraging potential applicants.

The following Table shows the number of grants awarded and the total amounts of funds expended (in millions) each year for the past five years.

	FY '71	FY '72	FY '73	FY '74	FY '75
Number	1,256	1,280	1,056*	1,065	1,221
Dollars	$53.6	$64.2	$62.4*	$70.0	$86.6

*Exclusive of impounded funds

During the first three years of this period the number of grants awarded decreased about 15 percent even though the amount of funds increased modestly. Presumably, this is due simply to inflation and the increased cost of doing research. In FY '75 the Institute was fortunate in receiving all of the funds appropriated by the Congress.

A portion of this Report is traditionally devoted to changes and problems related to the professional staff. The change that is uppermost in the minds of all of us is the pending "reorganization" in which all grant and contract activities are to be organized in four program areas, Communicative Disorders, Stroke and Trauma, Other Neurological Disorders, and Fundamental Neurosciences. An established senior scientist is to be selected or recruited to be in charge of each program area. It is said that under this arrangement each program will be more effective, more targeted or directed, more relevant, more responsive, etc. This is partially in contrast to the present system where applications are initiated by one, or small teams of investigators and hence the general direction of research is really determined by the scientific community at large and depends on research developments as they occur across a broad front. The present staff will without question cooperate in every way to make the new arrangement function as effectively as possible. However, it seems appropriate that their concern be recorded here.

There may be some major research developments (polio vaccine) in which management or "direction" played a significant role. In general, however, the role of research management is certainly debatable. There is no question about the essentiallity of basic research information. Also, it is generally agreed that basic research is intuitive to a large extent and cannot be successfully managed. Perhaps it is worth noting that it is not just recently that the National Cancer Institute has had an extravagantly financed and highly managed research program. A similar situation on a lesser scale has existed since 1958 when 50,000 compounds per year were being routinely and emperically screened against animal tumors and nationwide clinical trials were being carried out on every common type of cancer and every drug for which there was any conceivable rationale. And the results? Recent reports indicate that, except for a few rare and highly specialized types of cancers, the mortality from cancer has been reduced little, if at all in the last 25-30 years. The sad part about the heavy support of such "applied" research is that, if the desired results are not obtained, the work is essentially useless. In the case of basic research, immunology or virology for example, supported by cancer research funds, the results very likely will be useful to some area of research if not to cancer. The following quotation from the Sixth Annual Report of the Committee on Growth, National Academy of Sciences-National Research Council, 1951 is as applicable as ever:

> "The progress of science is not random. It has a strong sense of direction, intuitive but compelling. It is held to its course as though by an automatic pilot. By some mysterious telepathy, the thoughts of independent investigators mold each others emerging ideas and there develops a consensus of opinion that is the real architect of the pattern of research at any time. No committee, however wise, can substitute for this consensus. No wise committee would attempt to do so."

The reorganization of NINCDS Extramural Programs, the last Institute to follow this general plan, represents the end of an era in a very real sense. About 1958, largely through the efforts of NIH, the Civil Service Commission announced the availability of positions in an entirely new professional category, the scientist administrator. Prior to this time many people were doing this type of work, but they had to be classified as biologists, chemists or as some category of scientist, often to their disadvantage. The rationale for this new profession was simply that the ideal qualifications for the administration of research programs are a scientific background and experience and training in administration. Such a person need not, and could not be an expert scientist in the various areas in which he might work. Specialized scientific advice is much better obtained from consultants who are true experts in their respective fields. But such a person would understand the problems of the research scientist and would be familiar with the administrative mechanisms to facilitate his work.

This idea became highly popular and successful. No place else in the world is there such a wealth of expertise in science administration than at NIH and her sister health-related agencies. The unquestioned success and the excellent reputation of the extramural programs over a period of 20 years of logarithmic growth is due to a large extent to the expertise of scientist administrators. The idea was adopted widely by universities, medical schools, and large research institutions. The demand became so great that for more than 10 years NIH has been giving a course, the Grants Associates Program, in which up to 25 carefully selected junior scientists with an interest in, and a knack for administration spend a full year in on-the-job training in different parts of NIH and other agencies, Government and private, involved in science administration.

The reorganization of extramural activities on a highly programmatic basis, now essentially NIH-wide, leaves little place for the scientist administrator. Present members of the profession doubtless will be absorbed and given positions with limited opportunities to make important contributions. However, the program directors and their staffs will presumably be well established scientists with enough expertise to provide scientific direction to their areas of responsibility. The financial management and administration will be provided by people with no scientific background and usually little or no formal training in administration, but who have become familiar with Government regulations through on-the-job experience. The scientist administrator fits in neither category. In this plan he is neither fish nor fowl.

In the space of 20 years we have seen the rapid growth and dissolution of a profession. One might say this is just a sign of our times when many changes are occurring. For example, because science has moved forward so rapidly, obsolescence in the prime of life is one of the serious problems for some academic scientists who may have to almost completely change their field to keep abreast of the times. In these cases the reasons are quite obvious. In the case of the scientist administrators the reasons are less apparent. How have they failed? Have the programs been poorly managed? Has research stagnated? There is no evidence that this is so. Some Institutes have been organized on a programmatic basis now for several years. There is little or no evidence that their programs are any more effective. A primary reason is that really good research scientists do not want to dilute their talents with administration. Also, how are they to avoid very rapid obsolescence when they are completely isolated from the laboratory, the clinic, and the academic environment?

In the absence of a more convincing justification for the programmatic organization one is reminded of the following quotation from an article by Dr. William H. Stewart when he was Surgeon General of the Public Health Service and the Service was about to be reorganized into oblivion (so it was thought):

> "We tend to meet any new situation by reorganizing. And a wonderful method it can be for creating the illusion of progress while producing confusion, inefficiency and demoralization."

This quotation has an exceedingly comtemporary ring to it. However, those words were spoken by Petronius Arbiter, a Roman official of Nero's day about 200 A.D.!

The numbers of grants and the amounts of funds in the various disorder categories are shown in Appendix A.

FUNDAMENTAL RESEARCH IN THE NEUROLOGICAL SCIENCES

Introduction

The history of science shows clearly that improvements in the treatment of disorders are largely dependent on sound basic research. Nevertheless, support of basic research seems to be very much on the defensive in recent years. The popular demand appears to be for more applied, more directed, more targeted, and more responsive research. Those who favor basic research point out it is like a new born baby. One cannot predict what good it will be but its potential is great and it almost always is some use. Also, basic scientists are not doing research "just for the fun of it;" "just to amuse themselves." True, they enjoy doing research, but the reason for enjoying it is that it is a search for new knowledge. Without the hope of obtaining new knowledge, there would be little or no satisfaction in doing research. Therefore, the basic scientist should not be condemned for enjoying one of the most challenging vocations of all, the search for new knowledge.

The main elements of fundamental research in the neurological sciences usually include the synapse, the neuromuscular junction, neurotransmitters, and the nerve membrane. There are two rather new areas that are attracting increasing attention in recent years. One is the use of neurotoxins from venoms and various sources as highly specific probes to analyze nerve membrane structure and the function and structure of the synapse. This approach shows great promise and is a good example of basic research in a quite unrelated field (snake venons) making very important contributions to urgent medical problems. The other new area is the application of genetics to the determination of patterns of activity of a single specific neuron. Again, this approach is now possible as a result of half a century of research on mapping the genes in the chromosomes of the fruit fly.

Many other areas of important basic research could be described. A nerve growth factor has been discovered which, in extremely low concentrations, causes a remarkable growth in nerve fibers. Many efforts are being made to modify the shape or chemical composition of drugs and chemicals in subtle ways to try to make them more effective in therapy, to reduce their toxicity, and to develop chemical tools to elucidate cellular functions or structures. Examples are new and better anesthetics, drugs for the treatment of epilepsy and parkinsonism, and chemicals for analytical tools to study nerve membrane structure and other neurophysiological problems. These important approaches involve such sophisticated techniques as x-ray crystallography, electron paramagnetic resonance spectroscopy, and nuclear magnetic resonance spectroscopy. On the basis of such highly specialized information on the atomic interrelationships and the electron activity in the effective molecules, it will be possible to synthesize other compounds which are more effective or which play a crucial role in studies on how present drugs work.

The Synapse

The great importance and the almost infinite number (many billions) of synapses in the nervous system have only been hinted at above. Every nerve impulse crosses a synapse to pass from one nerve to another. The synapse must include structural and functional relationships to make it possible to pass an impulse in a few milliseconds and to pass many impulses per second. The synapse is not only the central figure in the "stop" and "go" action. It undoubtedly plays a crucial role in many more subtle activities of the nervous system which makes us the smooth working, coordinated organisms that we are instead of behaving like mechanical robots. That is, the synapse is a major factor in the placticity or flexibility of the brain which is essential to normal function.

The other half of the team that serves this highly important function of the nervous system is the neurotransmitter, the chemical that carries the nerve impulse across the synapse. In 1921 it was first shown that when a nerve was stimulated a chemical, in this case acetylcholine, was released at the nerve ending. It is now known that these neurotransmitters represent a common mechanism for transmitting an impulse or inhibitory or excitatory influences from one nerve to another or to a target organ. On the basis of the strict criteria that have been accepted only two compounds, acetylcholine and noradrenaline (norepinephrine), are now established as neurotransmitters. There are a number of "putative" transmitters, nerve chemicals that meet many, but not all of the criteria. They are dopamine, adrenaline, serotonin, octopamine, histamine, gamma aminobutyric acid, glutamic acid, aspartic acid and glycine. This list of probable neurotransmitters, which is doubtless still incomplete, is a further indication of the great complexity and amazing versatility of the nervous system.

Synapses also are especially important in any recovery of function after nerves are cut in the brain or spinal cord. As discussed elsewhere in this Report, nerve fibers can now be induced to regenerate under many different conditions. However, one of the major problems is the development of functional synapses with the appropriate recipient nerve cells. Why do synapses form when and where they do? It seems clear that the axon of a given neuron is directed to reach a specific part of the nervous system, and to form synapses on specific cells within their region, and on specific parts (soma or dendrites) of the neurons within the region. The way in which all of this is brought about is a great mystery.

One laboratory has approached this problem by studying synapse formation in tissue culture where the process is more readily observed and the conditions for growth are much more readily controlled than in a living animal. Bits of tissue from different parts of the rat embryonic nervous system were grown in tissue culture in different combinations about 1 mm apart. In some cases nerve fibers grew across the space and formed synapses in the neighboring piece. The results can best be summarized by the following simple table:

TISSUE	NUMBER OF SYNAPSES
Superior cervical ganglia (SCG) alone	2
SCG with cerebral cortex	3
SCG with thoracic spinal cord (TSP)	126
SCG with cervical spinal cord	34
SCG 5 days after TSP removal	4

Two pieces of SCG cultured together do not innervate each other or form many synapses. Similararly, cerebral cortex does not innervate nor induce synapse formation in the ganglia (SCG)even though cortex contains many neuron types. However, thoracic spinal cord (TSP) produced much innervation and synapse formation in the ganglia. Cervical spinal cord was in an intermediate position. Of special interest was the fact that five days after removal of the TSP the large number of synapses expected to be found had almost entirely degenerated. This proved that synapse formation was due to the nerves grown into the ganglia from TSP. This experiment suggests that membrane properties necessary for selective synapse formation are retained in neurons even after they have grown across an intervening surface in culture and have invaded an adjacent explant. As is often the case, the study raises more questions than it answers. Why does the cerebral cortex, with its many types of neurons, not produce innervation and synapse formation in the superior cervical ganglia? What is the difference between the thoracic spinal cord and the cervical spinal cord that the former is so much more active in this regard than the latter? These are problems which can now be attacked by this technique.

In another laboratory a study of these problems is being carried out on nerve cells in mouse and chick embryos. In Purkinje cells the excess of spines in the soma and dendrites of these cells in normal developing animals degenerate in large numbers within a few days after their sprouting. This does not occur in animals with agranular cortex whose spines remain healthy for weeks although they have never established synaptic contacts. It is suspected that the sprouting of spines in Purkinje cells is elicited by the climbing fibers. However, the sprouting may be a programmed phenomenon or it may be the result of the activity of basket cells.

EM studies with basket cells pursued further the findings of this laboratory that these cells also have spines arising from their somata similar to those observed in their dendrites and capable of establishing synaptic contacts. The disappearance of these transient processes, then, appears to be more of a question of remodeling than of regression after failure to establish successful contacts. It was found that both Purkinje and basket cells show a common pattern of proximo-distal development of spines.

The pontine cells (ventral pons) are unusual because in adulthood they lack, almost entirely, synapses on the cell body but have many on the surface of their dendrites. The investigators will try to determine whether spines sprout from the somata during development and will compare the pattern of development and morphological features of regular versus hook-spines, a feature of these cells in which the spine curves around large boutons and forms large synaptic contacts on their concave surfaces.

These last EM studies will set the stage for preparation of neuronal aggregates of simple composition in vitro, where pontine cells offer an attractive model. Such studies exploit for the first time the Golgi method for in vitro investigations. Spherical aggregates have been prepared from dissociated cells of chick embryo cerebrum, of chick chorioallantoic membrane, and of retina. They show the entire silhouettes with the shape and size of neurons, the pattern of branching and orientation of their dendrites, well differentiated cells, and "optimal" organization. The space available in aggregates is adequate for the cells to develop their basic dendritic patterns and the spherical form produces minimal distortion except that axonal length seems restricted. Axons appear short and branched. This new combination of EM and Golgi techniques is now ready to be applied to studies of synaptogenesis in vitro. It is expected to provide a great deal of new information.

Little is known about the mechanisms by which neurons establish specific connections with each other and with target cells in general. In the formation of neuromuscular junctions it is still unclear why only some nerve fibers form functional synapses or why some muscle fibers are innervated more than once and others not at all. Another investigator is studying this problem, also in vitro, using histological, EM and electrophysiological techniques. Embryonic chick ciliary ganglia were grown in culture with embryonic chick skeletal muscle. Nerve processes grow from the ganglia and contact the muscle fibers. Ciliary ganglia are used because the nerve cells in them are all very similar in that they are all cholinergic motorneurons which normally innervate the intrinsic muscles of the eye. This uniformity makes it easier to interpret the results.

By intracellular recordings from muscle fibers spontaneous depolarizing potentials were invariably observed and were identified as synaptic potentials and synaptically-evoked action potentials. In young cultures, the duration of the action potentials was very long, sometimes lasting several seconds. As the cultures matured, the action potential duration decreased. This was in agreement with the work of other investigators who also showed that the long duration "plateau" is maintained by an inward calcium current and the shortening develops so that the outward potassium current rapidly repolarizes the more mature fibers.

Spontaneous subthreshold potentials were also observed in the muscle fibers as early as 15 hours after the culture was prepared. They were reversibly abolished by curare (5 ug/ml) and all but the small potentials by tetrodotoxin (1 ug/ml). The simplest interpretation of these results is that neurons spontaneously generated action potentials which caused the release of relatively large amounts of acetylcholine.

Other workers have shown that with muscle and spinal cord neurons only 5-10% of the nerve processes that contact muscle fibers form functional synapses. Since the spinal cord preparation includes a variety of nerve fibers, many of the processes might originate from non-cholinergic neurons and hence could not excite the muscle fibers. However, even with the ciliary ganglia where the neurons are very similar, not every nerve-muscle contact is a functional one even though the muscle fibers may be contacted by many processes and innervated by several neurons.

Evidence of multiple innervation was seen in every muscle tested. It consisted of a synaptic response which was graded with stimulus intensity; the larger the stimulus amplitude, the larger the synaptic potential in the muscle fiber. Presumably increasing the stimulus simply stimulated more neurons in the ganglia. In summary, processes originating from several neurons form functional synapses with individual muscle fibers. These synapses form at different points on the muscle fibers, and muscle fibers have multiple end-plates and are multiple innervated.

There is still the question of why only a small proportion of the contacts are functional. Efforts were made to stimulate the nerve right where it made contact with the muscle. No synaptic response could be evoked even with intensities 10 times greater than used with functional synapses. Preliminary experiments have tested the tension required to detach the end of the neuron from the muscle. Some processes were easily peeled from the muscles. Others could not be detached, but broke or tore the muscle membrane. Bursts of synaptic potentials were often seen when stretch was applied to firmly fastened processes. They were never seen in non-adhering processes.

Fifty-three muscle fibers were mapped for acetylcholine sensitivity by applying ACh at 24 points about 10 microns apart. Localized patches of ACh sensitivity ("hot spots") were observed which consisted of about 5% of all points and which were 2.5-20 times more sensitive than background. No difference was observed between innervated and non-innervated fibers. Eighteen of the 20 hot spots found in innervated fibers occurred at points of nerve contact. These results suggest that developing muscle fibers have on their surface small patches of membrane which are highly sensitive to ACh. Unspecialized nerve processes grow from the ganglia and contact the muscle. Those processes which contact the preformed ACh "hot spots" are somehow induced to differentiate in such a way as to become capable of releasing transmitter at that point. An adhesion develops at that point between nerve and muscle, and the muscle membrane sensitivity to ACh further increases at the synapses. By such methods the mysteries of synaptogenesis will be clarified.

The Neurotoxins

Almost entirely within the past five years a completely new and exciting area of research has developed in the field of neuropharmacology. This is the use of toxins from snake venom, poison frogs, puffer fish, honey bees, and even bacteria as highly sensitive and specific tools to study nerve membrane structure, nerve transmission and synapse function. Some of these toxins have been mentioned in previous reports. However, they now play such an important role in research that they should receive more emphasis at this time.

The following list of toxins and sources probably is incomplete but it gives an idea of the number and variety used in research.

1. Australian Tiger Snake venom.
2. Batrachotoxin - arrow poison frog from Columbia.
3. Black Widow Spider venom.
4. Botulinus Toxin - Clostridium botulinum.
5. Bungarotoxin - *Bungarus multicinctus* (snake).
6. Histrionicotoxin - *Dendrobates histrionicus* (Columbian frog).
7. Melittin, minimine - crystallized peptides from honey bee venom.
8. Pumiliotoxin C - arrow poison frog from Columbia.
9. Tetanus Toxin - *Clostridium tetani*.
10. Tetrodotoxin - Japanese puffer fish.

Several investigators have suggested that the release of neurotransmitters to cross the synapse is stimulated by an influx of calcium into the synaptic terminal. One investigator has studied this mechanism in detail by applying the highly specific beta-bungarotoxin to the neuromuscular junction which is one type of synapse. After exposure to the toxin, there was an *initial* stimulation of both spontaneous transmitter (acetylcholine) release and release evoked in response to nerve stimulation. Simultaneous measurement of changes in delayed transmitter release led to the conclusion that the toxin increases the amount of calcium in the terminal during stimulation, either by extending the time during which calcium channels are open, or by reducing the rate of calcium removal from the terminal. Several hours after exposure to toxin, transmitter release fails, not by depletion of the terminal, but more probably by calcium accumulation. If free mitochondria and synaptosomes including mitochondria are exposed to the same concentrations of beta-bungarotoxin and then osmotically shocked, the fragmented synaptosomes retain their calcium uptake whereas the fragmented mitochondria do not. These results mean that mitochondria within synaptosomes are protected by a permeability barrier, presumably the plasma membrane, and thus provide a useful criterion for distinguishing free mitochondria from synaptosomes. These points are especially important because they play a crucial role in the transfer of calcium which in turn is important in the release of transmitter.

The production of action potentials (nerve impulses) in nerve can now be interpreted as being due to a specific increase in the permeability of the nerve membrane to sodium and potassium. One team of investigators has studied this mechanism through the effects of a number of toxins and drugs. It has found that tetrodotoxin (TTX), the puffer fish poison, produced a great increase in the permeability of the nerve membrane to sodium. The TTX became bound to the inner surface of the nerve membrane. By measuring the number of TTX molecules so bound, it was possible to estimate that there were 13 channels per square micron of nerve membrane through which sodium could pass.

Batrachotoxin (BTX), from the Colombian arrow poison frog, caused a marked increase in the permeability of the nerve membrane to sodium regardless of whether the toxin was on the inside or the outside of the membrane. However, BTX exerted no effect on the membrane potential when sodium was absent from the perfusion media on both the outside and the inside of the membrane. It was concluded that BTX specifically increased the resting sodium permeability. The ability of the nerve membrane to undergo transient sodium conductance increase upon stimulation was not affected provided that the membrane potential was restored with a polarizing current. It was concluded that there was no need to assume separate resting and active sodium channels in the nerve membrane. BTX also opens the channels that permit both sodium and lithium to penetrate the nerve membrane under resting conditions. TTX inhibits BTX action. Procaine also protects against BTX, but by specifically preventing BTX from binding to the receptors (macromolecules) at the target site in the membrane. Therefore, procaine, as well as TTX, may serve as an excellent tool for the isolation of BTX receptors which may be very important in the neuropharmacology of other toxins and drugs.

Another laboratory has also been studying the molecular structure and chemical composition of tetrodotoxin (TTX), which is presently used in the Far East to alleviate the excruciating pain of terminal cancer patients. It is among the most potent local anesthetics known but its clinical usefulness is limited by its toxicity and its tendency to diffuse rapidly away from the site of application. With highly sophisticated techniques of organic chemistry, promising steps have been made in changing the chemical composition and structure of the TTX molecule to retain the potent local anesthetic action, but eliminate the undesirable characteristics.

In addition to the extremely important practical aspects of this research, TTX is one of the most powerful chemical tools for investigation of the fundamental mechanisms of nerve impulse generation and transmission. Superficially, the action of TTX resembles that of local anesthetics such as cocaine and procaine in that it blocks the peripheral nerve and muscle conduction, as well as the central nervous system, without altering the resting potential of the nerve. At the molecular level, however, the action of TTX differs strikingly from that of other local anesthetics. The minimum effective dose is only 1/160,000 that of cocaine. While procaine and similar agents profoundly affect the conductance of both sodium and potassium ions, TTX blocks only the sodium current. Recent studies by other investigators indicate that TTX does not affect the kinetics of the sodium or potassium current whereas procaine delays the maximum in the sodium current and delays the rise in the potassium current. Procaine is equally effective when applied to the external or the internal surface of the nerve membrane, but TTX has no effect when applied internally. TTX does not block the acetylcholine-induced sodium conductance increase at the nerve synapses. Therefore, it is possible to separate the study of nerve impulse generation and conductance along the axon from the study of impulse transmission across synapses by comparing the action of TTX with the action of curare, for example, which prevents nerve conduction across synapses.

These organic chemists have synthesized numerous derivatives and intermediates of TTX. One approach began with shikimic acid, a readily available natural product which there is reason to expect can be converted to active TTX derivatives. The investigators have been fortunate in obtaining 200 milligrams (1/150 oz) of the rare natural TTX from Japan. They are learning a highly sensitive technique for assaying TTX activity although the essential, close collaboration with several neurophysiologists will continue. The derivatives of TTX which have been prepared without both a two-thirds ortho ester moiety and a guanidinium moiety are almost completely devoid of activity. Therefore, it seems that these two parts of the molecule are essential. Attempts are now being made to incorporate a paramagnetic nitroxide spin-label and a fluorescent probe into an active derivative to make spectroscopic studies of the molecular structure of TTX as the molecule "sits" on the nerve. Such studies will provide crucial information about the most detailed structure of the nerve membrane and the mechanisms by which neuroactive drugs function.

The penultimate step in this exciting development just occurred in June 1975 when the Institute began supporting a modest research program aimed at the total synthesis of the frog alkaloid histrionicotoxin. Because these toxins are so highly specific in their neuropharmacological activity, it is essential to know their atomic composition and molecular structure to the last detail. Even very minor changes in these factors may markedly change or completely destroy their activity. One approach to this problem is the chemical modification of the toxin obtained from natural sources as described above. This is a useful technique and sometimes the only one available with very large, complex molecules. However, because the entire molecule is not known, there is always some uncertainty about the chemical basis of its action. A much more accurate method is the total synthesis in which the investigator starts with known chemicals and synthesizes the molecule from "scratch." In this way the complete structure and composition of the molecule is known, the atomic groups responsible for its activity can be readily identified, and the structure and composition of the molecule can be modified in systematic ways to change its activity. Through such steps it is possible to show exactly how the molecule acts on the nerve and, in turn, gradually learn exactly how the nerve works.

The other arm of this exceedingly important tool also has developed mostly in recent years. This is the field of neurotransmitter substances. There is now a large number of such substances including acetylcholine, noradrenaline (norepinephrine), dopamine, adrenaline, serotonin, octopamine, histamine, gamma amino butyric acid, glutamic acid, aspartic acid, and glycine. A great deal is known about how these compounds function in nerve transmission. With a great variety of highly specific toxins as probes for certain types of molecules in the nerve and with many neurotransmitter substances, changes in which may assist in determining the specific action on the nerve, there surely will be rapid progress in analyzing the molecular structures and functions of nerve cells. This is an excellent example of two branches of a science developing quite separately and independently and coming together to focus on a common problem?

Genetic Determination in the Nervous System

It is an exciting privilege to watch during a period of only a few years a fundamental science of the "purest" sort come to bear on some of the most dreadful health disorders; in fact to develop a whole new health science. For well over 50 years geneticists have been patiently mapping the genes on the chromosomes of the common fruit fly, Drosophila melanogaster. They received little encouragement other than from their own colleagues. The "applied" scientists were inclined to take the "so what" attitude. Who cares whether a fruit fly has a brown eye or a white eye; or how many bristles there are on its abdomen? "Basic" scientists just do research for their own amusement. It is unlikely to have any practical use! However, this whole fund of information has formed a foundation for exceedingly important research on how genes control the development of, and deficiencies in the nervous system.

One investigator points out that the gene-symptom relationship must be investigated directly in order to solve the problem of genetic disorders of the central nervous system. By far the best material on which to make such studies are the neurological mutants of D. melanogaster. Four single-gene mutants expressing specific leg movement disorders have been produced in Canton-S stock and have been located on the X chromosome. All four of these mutants express leg-shaking disorders under the influence of ether, with patterns specific to each gene. Studies of the neural mechanism underlying the leg-shaking disorders have already shown that the phenotype disorder is expressed autonomously in certain neurons in leg motor regions. The activity pattern of each neuron is analyzed by a digital analyzer for quantitative description. Based on this analysis, the possible functional model of the activity center for the disorder at each leg motor region is constructed.

The specific pattern coded by a gene can be modulated by the recombination of another gene. With this dual-gene mutant, the pattern formation mechanism is studied in further detail. The Eag gene has a particular action to release the disorder coded by other genes. The release mechanism of the disorder is investigated with dual-gene mutants with Eag. Information thus obtained will elucidate the physiological mechanism of the genetic disorder in the motor system and the release mechanism of the genetic code stored in the neuron.

The motor nerve activity specific to the leg shaking induced by a single gene mutation is the bursting discharges of characteristic pattern. Each neural unit composing a burst was determined by recording the activity in single motor neurons in the thoracic ganglion. It was found that the total number of motor neurons involved in leg shaking in the Hk^1 mutant must be seven. In another mutant (Sh^5) the same motor neurons seemed to be involved and the difference in pattern must be dependent on the difference in a pacemaker neuron. In order to study the mechanism by which genetic information is decoded at a single neuron and controls motor function, this study has taken one large step by revealing the activity pattern of each of seven motor neurons composing the bursting discharge responsible for the leg shaking. Study of the phase relationship among activities of those motor neurons will permit construction of the physiological model of the system. These data are useful not only for understanding each single gene mutant, but also for work on the next step which concerns a dual gene mutant of those two genes. With these methods, the entire system whereby genetic information is transduced to a neural code will be disclosed. This system will be the first neural system in which the transduction of genetic information to the neural code is directly studied. Thus, it not only opens up a new field of neurophysiological genetics, but also provides a model for the study of neurological disorders of genetic origin.

Gynanders and gynandromorphs! Sex determination in *D. melanogaster* is based on the ratio of sex chromosomes to autosomes. An individual with a single X chromosome is male, with two X chromosomes it is female. Occasionally during mitosis in early embryonic stages one of the female (X/X) cells loses one X chromosome and so becomes male. All of the descendents of this cell are male, but all of the descendents of the other cells are female. Therefore, such individuals contain both male and female tissues and are called gynandromorphs. Spontaneously arising gynanders are rare. However, techniques have been developed to increase their frequency. This research was reported in 1919, but only recently have new techniques made it applicable to the development of the nervous system.

The use of gynandromorphs in research requires that the sex (genotype) of the cells to be studied be known. In the case of many parts of the body (cuticle color, bristles, eye color, etc.) the appearance of the organ will indicate the sex. However, there were no such "markers" for the nervous system until a couple of years ago when two young postdoctoral students developed a series of markers to detect the genotype of various internal organs, including the nervous system. The technique involves the histochemical detection of enzyme acid phosphatase activity. The genetic locus that controls the enzyme activity is on the X chromosome that will be lost in the formation of the gynandromorph. The other "doses" of this gene contain alleles which code for protein with little or no enzyme activity.

Two types of experiments are now possible. A detailed embryological "fate" map of the nervous system can now be made. This will show from which embryonic cells various parts of the nervous system come. A similar map may be made of the larval nervous system. Comparing these maps will give information about the rules which govern transition from one to the other. A second type of experiment is to use the gynandromorph itself as a culture vessel to study the interaction of mutant and normal tissue *in situ*. For example, one can create an individual in which the eye is of one genotype and the optic lobes of another. Mutants are already available which perturb the formation of connections between the eye and the optic ganglia. Using these, it is possible to determine which of the elements, presynaptic, postsynaptic, or both, are responsible for formation of normal connectivity.

The fate mapping of the adult nervous system has been completed and the mapping of the larval nervous system is nearly finished. Virtually all 70 of the known loci that produce abnormalities in the eye have been screened. Only 7 have also given rise to abnormalities in the projection of retinula axons to the optic genglia. There were no changes in other parts of the nervous system. Somatic recombination experiments with these 7 mutants has shown that 1) mutant retinula cells contacting wild-type optic ganglia lead to distortions in the optic ganglia typical of the mutant; wild-type retinula cells contacting mutant ganglia lead to normal organization in the ganglia; 2) normal alleles at the two loci are functioning before mid-second larval instar, well before actual physical contact between retinula axons and lamina. A number of generalities concerning which cells divide in the larval nervous system and where they reside in older larvae and adults already have been defined. It is clear that genetics is a potentially powerful tool in the analysis of the development and functioning of the nervous system.

With the development of modern techniques it is now possible to study the influence of genes directly on nerve activity in invertebrates. The control of autogenic activity of neurons is performed by interneurons, that is "command fibers." The system which is composed of an autogenic center and command fibers may well be a basic working unit in the central nervous system. The important point is that the activity pattern of the autogenic center is genetically coded. The role of the command fiber (interneuron) is to trigger the activity of the center which is genetically programmed. Therefore, the observation of the patterned activity of the center is, in fact, the observation of the genetic code inherited in the neuron. Here the genetic code reveals its phenotype as a pattern of nerve impulses. Therefore, the neurophysiological study of the patterned activity of an autogenic center will interpret the neural activity in terms of genetics. In fact, this investigator has already shown that specific neural activity caused by a single gene mutation is detectable in the central nervous system of the house fly and it is autonomous in the genetic sense in that neuron. It is now clear that the genetic technique will contribute a great deal to the study of synaptic function for coding in the central nervous system.

A study of peripheral synaptic transmission has been carried out on the dorsal longitudinal flight muscle of the house fly, Musca domestica, and the blow fly, Calliphora erythrocephala. The anatomical arrangement of this muscle is such that a single muscle fiber can be identified in each experiment and each fiber is innervated by a single excitatory axon from the posterior dorsal nerve. No inhibitory innervation was found. As a side point, it is very interesting to note how sensitive the synapse (neuromuscular junction) was to decreased oxygen tension as shown by measuring the electrical response of single muscle fibers after stimulation of the nerve. The postsynaptic potential became slower and smaller before any change in resting membrane potential occurred. Synaptic transmission was blocked even when the electrogenic response of the muscle membrane and the nerve membrane were unaffected. This showed the great importance of maintaining the oxygen tension in all experiments.

An excess of magnesium ion reduced the amplitude of the postsynaptic potential without changing its time course. The reduction was proportional to the concentration of magnesium. In comparison to the vertebrate neuromuscular junction, this junction was quantitatively less sensitive to magnesium but appeared to have the same basic mechanism to reduce the quantum contents. The postsynaptic potential was also reduced in amplitude by a reduction in calcium concentration. The postsynaptic potential was extremely sensitive to changes in calcium concentration as compared to changes in magnesium concentration. As was to be expected, calcium ion quantitatively antagonized the effect of magnesium ion, but the great difference in sensitivity suggested that the site of action of these two ions might be different rather than that it was a simple antagonism.

In the hyperkinetic mutant (Hk1P) of D. melanogaster in which spasm-like leg movements are due to a change in a single gene on the X chromosome, as described earlier, it is likely that one of the enzymes necessary for the synthesis of a transmitter has been eliminated by the mutation, while other enzymes related to the structure of the nervous system were unaffected, because the behavior of the mutant is almost normal. Motor centers for the spasm were isolated in the thoracic ganglion and the rythmic activity was found to be endogenous in the thoracic ganglion because the activity remained unchanged even after total deafferentation or complete isolation of the ganglion from the rest of the nervous system. Therefore, the genetic change is autonomous in the thoracic ganglion.

The motor neurons responsible for the leg shaking spasms were located in the three pairs of motor regions in the thoracic ganglion. Their rythmic activity was endogenously (genetically) patterned. Besides these motor neurons, pacemaker-type neurons (interneurons) which controlled the activity of the motor neurons at each region were found. The further relationship between motor and pacemaker neurons is still being investigated.

CEREBROVASCULAR DISORDERS

Throughout the last 15 years, the NIH (NINCDS) has concentrated its support of stroke research in the areas of the pathophysiology, diagnosis and treatment of cerebrovascular disease. There has always been an effort to look at the new, the old and the neglected problems in the field of cerebral vascular disease. Over the years, however, there has been a shift in research enthusiasm from studies in cerebral blood flow, stroke predictors, platelet adhesiveness to biochemical alterations during ischemia and discrete changes in the vascular wall during the evolving ischemic process. This is not to say that studies in cerebral blood flow have been less productive in yielding answers about the mechanisms of stroke or its treatment but each year or so a new emphasis in stroke research evolves. The support level has risen steadily over the years to $4,914,812 in FY 75 (15 research grants, 16 clinical research centers and 11 stroke acute care research units).

A number of laboratories using different approaches have attempted to shed light on the mechanisms of vascular control in the brain. These laboratories have used techniques which require the sophisticated measurement of regional cerebral blood, the precise measurement of neural transmitters, the use of the EMMI scanner or the application of the electron microscope for morphologic studies. One group studying the regulation of brain microcirculation by central noradrenergic neurons and using the Falck-Hillarp histofluorescence technique for the measurement of brain catecholamines have demonstrated that noradrenergic nerve fibers innervate the small intraparenchymal brain blood vessels, including capillaries. These neurons originate in the brain stem and ascend within the neuraxis to the blood vessels. This system appears distinct from the noradrenergic system which innervates the large, extraparenchymal cerebral arteries and veins. To investigate the role of this central noradrenergic system in the regulation of the brain microcirculation, the cerebral blood flow and the transcapillary transport of labelled water in monkeys were studied. The animals had been chronically sympathectomized and the system was manipulated by the instillation of short-acting alpha-adrenergic blockers. The results seemed to demonstrate that this central noradrenergic system decreases cerebral blood flow while increasing the capillary permeability to water. Blocking of the system has the opposite effect.

Evidence for the cholinergic neurogenic control of cerebral blood flow (CBF) has been provided by another Center. This group, using baboons, have measured CBF, autoregulation and the responsiveness of the cerebral circulation to carbon dioxide before and after the infusion of atropine sulphate, neostigmine or acetylcholine injected by intravertebral or intracarotid routes. Atropine infusion limited the increase in CBF during hypercapnia and enhanced the decrease in CBF during hypocapnia. Neostigmine had no effect during normal conditions but the intracerebral and intracarotid injection of neostigmine reduced the vasoconstrictive response to hypertension and produced no change during hypotension. Significant increases in CBF and cerebral oxygen consumption were recorded with acetylcholine infusion. Taken together, these drug studies provide evidence which seems to favor a functional role for cholinergic innervation in the regulation of CBF.

Another group of investigators has carried out animal studies to investigate the role of prostaglandins in cerebral vasospasm. Since the demonstration that introduction of blood platelets into the subarachnoid space of animals could produce cerebral vasospasm, many substances have been suggested as being involved. One from this group or class of substances studied are the prostaglandins ($F_{2\alpha}$ and $PGF_{2\alpha}$). It has been suggested that these substances can be produced at the vascular hemorrhage site by platelets released at the damaged vessel site. This team has presented data which indicates that there is an increase in $PGF_{2\alpha}$ in dog cerebral spinal fluid (CSF) when blood platelets are present, as well as increased levels of $PGF_{2\alpha}$ in the CSF of patients having intracranial hemorrhage. The addition of platelets to dog CSF increased the concentration of $PGF_{2\alpha}$. Preliminary experiments in humans also have indicated that the levels of $PGF_{2\alpha}$ are increased in the CSF following cerebral hemorrhage and the levels appear to correlate well with the degree of spasm. It would seem, therefore, that these data lend credence to the hypothesis that the prostaglandins may play a role in the etiology of cerebral vasospasm, at least in the acute phase.

In a series of normal young men, the cerebral blood flow was measured by radioactive xenon inhalation both in the resting state, during movement of one hand and during spontaneous speech. These studies revealed significant CBF increases in the region classically associated with these functions. Similar studies were carried out in patients with hemiparesis and aphasia in pursuit of the question, "Does the damaged brain try harder?" These investigators came up with two interesting conclusions: in some of these patients, the effort to carry out a function which is impossible or very difficult, e.g. to move the hand in the presence of hemiplegia, sometimes causes a decrease in CBF in the region of that function, or in the whole brain; and in some patients recovering from aphasia due to a *left* hemisphere lesion, significant increases in CBF were seen to occur in the *right* hemisphere during the effort to use word symbols.

The value of the development and utilization of the noninvasive xenon method, inhalation of the radioactive gas, has demonstrated its usefulness in the study of patients who had repair of their internal carotid arteries to correct the narrowing of the vessels. The progressive diminution of the lumen of these vessels is the result of a deposition of fatty plaques. Therefore it is hazardous to measure cerebral blood flow by the injection of the radioactive gas via the internal carotid whereby a plaque may be dislodged and carried up to the smaller vessels with the end result of occluding the vessels. The investigator who developed this technique studied 25 patients who had corrective surgery (carotid endarterectomy) with the hope that the surgery would increase the cerebral blood flow.

Gray matter (fast compartment) regional cerebral blood flows (rCBF) were obtained with the above method on 34 young control subjects and in 25 patients with severe stenosis who had undergone carotid endarterectomy. Using normal control data as a basis for interpreting patient results, an assessment was made of postoperative rCBF changes relative to the preoperative findings. Significant increases in rCBF were found in 11 of the 25 patients;

13 showed no change; one had a decrease in flow. Five of the 11 patients revealed greater changes in the hemisphere ipsilateral to the side operated on; the remaining had bilateral symmetrical changes.

When the CBF is reduced below the minimal values required to maintain normal oxidative metabolism, the energy state changes precipitously and drastically. Investigators in another Stroke Center have studied the energy state of ischemic monkey brains with the hope of understanding the mechanisms of neuronal damage. Reduced nicotinamide adenine dinucleotide (NADH) fluorescence was recorded from an avascular area in the monkey cortex prior to, during and after focal incomplete ischemia. In normal cortex, NADH remained constant throughout a wide range of variations in blood pressure and $PaCO_2$. In ischemic cortex NADH levels were higher in hypovolemic hypotensive animals than in normotensive normovolemic animals. Neither hypercapnia nor hypocapnia were effective in decreasing NADH in regions of ischemia, but the latter was associated with a degree of hypotension which interfered with the interpretation of data. NADH returned to normal with restoration of flow, supporting the reversibility of this degree of ischemia. The high levels of NADH at death, compared with the ischemic levels, were consistent with incomplete ischemia in this model of cerebral infarction.

Another study from one of the Stroke Centers has tested the hypothesis that cerebral ischemia may change the kinetic parameters of an ATPase enzyme requiring Mg^{++} and consequently the rate and amount of released transmitter. The results of these investigators' studies on the ischemic gerbil brain indicated that there is a positive correlation between the duration of ischemia and the decreased ability of this ATPase to hydrolyze substrate, with a concomitant decrease in transmitter release. In addition, they found that the significant decreases in activity occurred when lactic acidosis accompanied the cerebral ischemia.

Since glucose is the primary food substance of nerve cells, studies which perfect the measurement techniques of recording the brain's utilization of this substance will help to give the physician better insights about the brain's functional status. An autoradiographic method has been developed for the quantitative measurement of local cerebral glucose utilization in vivo using trace amounts of C^{14} deoxyglucose. This method is based on the fact that deoxyglucose is phosphorylated and remains in the brain for a relatively long time and that the rate of phosphorylation can be used as a measure of the rate of glucose utilization. These investigators found that the glucose consumption was 2-4 times greater in gray matter than white matter. Some structures, notably the inferior colliculus, had rates as high as six or more times that of white matter. In addition, glucose uptake was reduced as the level of anesthesia was increased.

The development of the scanning electron microscope (SEM) has given the medical community a biological tool to investigate in a precise way the early vascular changes which may develop during high fat diets or very brief periods of ischemia. Alterations in arterial endothelium have been considered to be of importance in atherogenesis. Since the SEM is ideally suited for the analysis of biological surface phenomena, it has been used in a study of early and perhaps initial endothelial changes in experimental atherosclerosis.

Minature pigs on an egg yolk diet had their serum lipids and testosterone levels monitored and were then sacrificed shortly after puberty. Preliminary results with the SEM have shown localized alterations of the endothelium which consisted of disorganization of the normal surface architecture and evidence of cell damage--restricted to certain sites near the ostia of intercostal arteries in the aorta.

Additional studies on the morphological changes in cerebral neurons following ischemia were carried out in another Stroke Center. This group of investigators clamped the right common carotid arteries in anesthetized rats ($PaCO_2$ maintained at 35-40 tor and the PaO_2 at 21 tor). Bicarbonate was infused to counteract systemic acidosis and to minimize variables. Animals were excluded when the blood pressure fell below 100 tor. After periods of 2-30 minutes, the clamps were released and the rats reoxygenated for 1 to 120 minutes. None of the six rats exposed to up to 5 minutes of hypoxia-oligemia developed neuronal abnormalities, whereas 18 of 29 rats exposed to ten or more minutes showed changes. Longer exposures resulted in more widespread neuronal damage and behavioral abnormalities developed. The presence or absence of neuropathological and behavioral abnormalities were concordant in 16 of 18 rats. No-reflow developed in only one of 24 animals examined after 20 and 30 minute exposures. The production of neuronal and neurological abnormalities in the absence of hypotension or no-reflow reemphasized the primary vulnerability of neurons to hypoxia and ischemia.

Over the last few years there has been an intense interest in the mechanisms of thrombus formation and its possible relationship to the genesis of thrombotic strokes. Investigators in one Center have investigated the association of cerebral infarction with local intravascular coagulation. The plasma factor XIII (fibrin stabilizing factor) after activation by thrombin, crosslinks fibrin fibrils of thrombi or fibrinous deposits and is consumed: its concentration in serum is only approximately 5% of that found in plasma. Therefore, since this factor has a low synthesis rate, its plasma concentration is reduced when intravascular coagulation occurs. In addition, the plasma concentrations of high molecular weight fibrinogen complexes are substantially increased in patients with either intravascular coagulation or thrombosis. Serum levels of both of these factors were studied in 48 cerebral thrombosis patients. Increasingly severe degrees of neurological deficit were found which were associated with greater declines in factor XIII concentration and greater increases in high molecular weight fibrinogen complex concentration. These findings seem to suggest that the pathophysiological reactions, consequent upon infarction of brain tissue, results in activation of the blood coagulation system, presumably at this local site.

Another group investigating the relationship of TIA's and aggregation of platelets outside the brain (which may later embolize the brain) have found that TIA patients have platelets which are hyper-responsive to ADP. About half of the 23 patients are also hyper-responsive to epinephrine. These patients are being given sulfinpyrazone (hopefully to decrease platelet reactivity) but the significance of drug treatment cannot be tested until the study is completed and the code is broken.

Both hypoxemia (a reduction of the arterial pO_2) and ischemia (a critical degree of hypoperfusion of the brain tissue) can deprive the central nervous system cells of the oxygen supply which they require to function normally. Therefore consciousness is lost when the arterial pO_2 is reduced to about 30 mm Hg or when the cerebral blood flow falls to about 50 percent of normal. These observations and others have led to the view that hypoxemia and ischemia share equally the potential of inducing brain anoxia and that irreversible anoxic damage may be precipitated by either. More recent information has indicated that there is a fundamental difference between pure hypoxemia and ischemia and that permanent cell damage is probably almost always caused by ischemia.

Epidemiological studies on the geographic variations of stroke mortality within North Carolina have shown that there are regional differences in stroke mortality rates in white men; the highest rates in the plains (tobacco growing and farming) area and lowest rates in the Smoky Mountain range. Analyses of underlying and contributory causes of death of white men aged 35 to 69 in 1969 showed age-adjusted stroke mortality rates of 200 and 119/100,000 in the Plains and Mountain regions respectively, i.e., a ratio of 1.7, a figure larger than that for different sections of the United States. Similar ratios were noted between the Plains vs. Mountain regions for cerebral hemorrhage and for cerebral infarction. Mortality rates from hypertension and diabetes were also higher among stroke deaths in the Plains than in the Mountain regions, suggesting that the presence of these risk factors may be the explanation for the observed geographic variation in stroke mortality.

The analysis of clinical data of a population of individuals with TIA-like symptoms by another group of investigators have shown that persons with no prior disease before having their TIA-like symptoms had mortality rates which were lower than those persons having had some disease state, e.g., high blood pressure and/or ECG abnormalities. These groups also had a higher proportion of current smokers. No differences were noted in serum cholesterol, hematocrit or ponderal index.

CENTRAL NERVOUS SYSTEM TRAUMA

Head Injury

Important strides have been made recently in improving our understanding of the pathophysiology of head injury. Much has been learned, both in laboratory and clinical investigations concerning the brain's response to insults such as anoxia-ischemia, elevated intracranial pressure, and trauma. Studies of alterations in cerebral blood flow and intracranial pressure, and of the brain's metabolic response to injury are providing information which may make improved treatment of the insulted brain more of a reality.

Currently the NINCDS is supporting five Head Injury Clinical Research Centers as well as fifteen related research projects.

At one of the Centers a Head Injury Clinical Research Unit was opened officially in March 1975. Patients admitted to the unit include those with acute head injuries and various other types of acute cerebral insults, acute stroke patients, and patients with multiple injuries including head injury.

Continuous recordings of intracranial pressure have been obtained from approximately 40 patients since the unit was opened. The data from these patients are being collated with that from another group of 150 patients in whom intracranial pressure was recorded in the surgical intensive care unit the previous three years.

Elevated intracranial pressure is being treated with continuous ventricular drainage, hypertonic mannitol, steroids, hypothermia, and most recently barbiturate anaesthesia.

Cerebral hemodynamic studies are essential for understanidng the pathophysiology of head injury and for guiding treatment and patient management. Since an immediate decrease in blood flow is a striking result in several types of head injury, a convenient, safe, non-traumatic, non-invasive technique for estimating cerebral blood flow in these critically ill patients would be of inestimable value. In an attempt to meet this need, investigations are being carried out on the xenon inhalation technique. This method which involves a one minute inhalation of xenon-133 followed by extracranial monitoring of its clearance can be safely and repeatedly administered to both patients and normal control subjects.

The widespread interest in non-invasive techniques is manifested by the present or planned use of the Xe^{133} inhalation method in three Head Injury and six Stroke Centers supported by NINCDS, plus nine additional laboratories here and abroad. Several other institutions have pursued the closely related technique of intraveneous Xe^{133} injection. A recent conference on non-invasive cerebral blood flow methods held at Duke University (September 1974), and attended by over 70 investigators, further attests to the current interest in such an approach.

In head injured comatose patients, hypertonic mannitol increases cerebral blood flow independent of changes in intracranial pressure and whether intracranial pressure is normal or elevated prior to administration of mannitol. The results suggest that even diffuse edema might reduce cerebral blood flow independent of intracranial pressure. This would be very important if it were true, because in clinical practice hypertonic solutions are not generally used unless there is evidence of intracranial hypertension, and in patients in whom intracranial pressure is measured continuously. The end point of treatment is reduction of pressure to or near normal. To test this hypothesis triethyl tin poisoned rats were used as the animal model. Results of these experiments confirmed the fact that edema per se can reduce cerebral blood flow independent of intracranial pressure.

Disturbed cerebral function in regions at some distance from the location of mechanical brain damage is well recognized. Also, head injury is often accompanied by subarachnoid hemorrhage and by the release of various substances into the subarachnoid space. The hypothesis has been advanced that vascular spasm and impaired cerebral blood flow following traumatic head injury are due to the release of one or more substances in response to the insult. It has also been postulated that these substances might interfere with neuronal activity independent of an effect on blood vessels.

In an effort to identify chemical agents that might be responsible for cerebral dysfunction following head injury, a series of experiments has been carried out in which individual biogenic amines were perfused over the surface of the brain of cats and their pathological, behavioral, and vascular effects studied.

The implications thus far are that norepinephrine produces a type of brain damage that is manifested by gross electrical dysfunction and brain swelling and that these changes are independent of an alteration in local blood flow at the site of the norepinephrine infusion.

A head injury model in the monkey which will more accurately reproduce the lesions observed in humans is being developed using the Hyge, an air cannon that provides 40,000 pounds of thrust and is capable of accelerating the monkey's head up to 2,000 g's. This project is a cooperative program with the Department of Engineering at the University of Pennsylvania. Measurements of cardiac and respiratory status, regional cerebral blood flow and metabolism, intracranial pressure, brain electrical activity, morphology, and a number of other parameters are being made in an attempt to determine the contribution of ischemia and hypoxia to irreversible brain damage in acceleration head injuries.

The brain, like any other organ, reacts to physical injury by swelling in its acute stage, and by formation of a scar as an aftermath, which frequently leads to the development of seizures. In fact a great many penetrating injuries of the head result in post-traumatic seizures within three years. Aside from the symptomatic treatment of this complication with anticonvulsants, a more rational but unorthodox approach would be to attempt to prevent the scar formation at an early stage, or to attempt to dissolve it (probably with an antiserum) after its formation. The success of the latter approach hinges on a clear understanding of the pathophysiology of gliosis, since scar formation in the brain results from astrocytic proliferation. A partially purified astrocytic maturing factor has been obtained from rat and pig brain. Although a 400-fold purification of this protein has already been achieved, further purification is needed for the purpose of chemical characterization. The feasibility of immunosuppression of gliosis and scar formation using antiserum against this protein is being explored.

Proton beam radiography is being developed to meet the need for a non-invasive technique for establishing accurate anatomic diagnosis in head trauma. The characteristics of the proton beam make it relatively insensitive to the calcium barrier of the skull and relatively sensitive to small differences in soft tissue such as those between brain and fluid or between gray and white matter thus providing optimal contrast.

Using this apparatus it has been possible to image the ventricles within an autopsy brain without displacement of the ventricular fluid. Blood clots and tumors may be seen in intact brain. The distinction between gray and white matter and between normal brain and multiple sclerosis plaques can be identified in brain slices. A brain tumor within a brain has been imaged within the skull. All of this has been done using the simple Polaroid film with minimal radiation dosage. None of this can be done using conventional x-rays and Polaroid film or by using acceptable radiation dosage.

Another diagnostic technique of interest involves ultrasound instrumentation to detect and localize intracranial hemorrhage and to differentiate it from cerebral edema. It has been demonstrated that the technique can be used to great advantage in the visualization of the intracranial contents of children three years of age or younger through the intact skull and through the intact skull of some adults.

A collaboration between physical scientists and clinicians has been instituted to provided an easily utilized, partially portable ultrasound diagnostic instrument which will view the entire brain in either coronal or horizontal section. The immediate oscilliscope readout will permit scanning of the entire brain in a few minutes. Permanent photographic presentations can be then limited to the important areas only. This procedure will decrease the time required to collate and study the multiple Polaroid photographs needed previously and will make the clinician's, and the technologist's task easier and more productive in a shorter scanning time.

More emphasis is being placed on reviewing head injuries in infants and young children. The method is expected to provide some information not available on angiography, with the possibility of scanning the brain much more frequently during the few critical hours immediately after injury than is possible by radiographic technics. Ultrasound scanning is not expected to replace angiography, following head trauma, but it is expected that it will add additional reliable information.

In cerebral hypoxia (restricted supply of O_2 to tissue) and ischemia (reduction in volume of blood flow) the common denominator is the decrease in cellular oxygen concentration. When sufficiently severe, or sufficiently prolonged, this cellular oxygen lack leads to derangement in cerebral metabolism which, by unknown mechanisms, eventually causes cell death. From a clinical standpoint it is important to define the pathophysiological mechanisms that effect oxygen transport to the cells in the most prevalent forms of hypoxia and ischemia. A meaningful study of cerebral hypoxia and ischemia must therefore include careful analyses of both physiological and biochemical variables, and correlation of the results with the neuropathological picture. Before any therapeutic manipulation is instituted (e.g. hypothermia, administration of anesthetics) the effects of these manipulations upon the normal brain must be carefully evaluated. Finally, since the pathophysiological factors affecting oxygen transport to the tissue are different in all the various conditions that are lumped together in the terms "hypoxia" and "ischemia", each condition should be studied separately.

Techniques have been developed in Sweden for measuring cerebral blood flow and energy metabolism in small experimental animals and for producing hypoxia and ischemia which can be graded and carefully controlled. These techniques include quantitative measurements of blood flow and oxygen consumption in rat cerebral cortex and whole brain. The metabolism of organic phosphates, glycolytic substrates, citric acid cycle intermediates and associated amino acids (in some instances also of neurotransmitters) has been measured during, as well as following, total ischemia of 1-15 minutes duration, and in hypoglycemia. Cerebral blood flow and oxygen consumption, as well as tissue metabolites, have been measured in hypoxia, hypercapnia, hypocapnia, hypothermia and anesthesia. The results of these measurements have allowed a detailed description of circulatory and metabolic events, characteristic of each of these conditions and have led to an appreication of the brain's rather striking resistance to hypoxia leading to the hope that this native resistance can be enhanced by therapy.

Spinal Cord Injury

Conventional thinking about spinal cord injury has been that spinal function is immediately and permanently lost following traumatic disruption of the cord. However, many years of laboratory studies with experimental spinal cord trauma conducted by universities and medical centers throughout the world now suggest that even severe physical injury does not always destroy the spinal cord. In some cases it has become evident that all of the tissue damage may not be directly related to the trauma, but may be an indirect result of it. Today, many scientists believe that the injury initiates a process of necrosis that eventually does destroy the cord. Within a definite time lag before this process is fully developed, the long fiber pathways are mostly intact. This fact raises the possibility that if this response of the tissue to injury could be stopped in the acute stage, then useful spinal cord function could be preserved and paralysis prevented.

To take advantage of new developments and to stimulate increased related research, the NINCDS is supporting six acute Spinal Cord Injury Clinical Research Centers. The aims of the Centers are twofold- experimental and clinical. This two pronged approach is entirely interrelated such as basic experimental observations are made, correlation with the clinical situation is attempted, each step therefore potentially resulting in improvements in the care of the patient with spinal cord injury.

The Centers also have a responsibility for community research on problems such as roadside diagnosis and emergency therapy, rapid transportation to the Centers, emergency room differential diagnosis, and patient handling. Proper handling during these phases of injury may well prevent lasting damage to the spinal cord. Additionally there are 24 related research projects which are currently active.

Results of electrophysiological studies at one of the Centers have shown for the first time that there is excellent correlation of the evoked potential measurement with the clinical state of the patient. By repeatedly stimulating a peripheral sensory nerve with a mild electrical shock one can record from the scalp a summated response whose latency, amplitude, and wave form configuration provides valuable information about the functional integrity of anatomical pathways in the spinal cord. The technique seems ideally applicable to the patient with spinal cord injury where the first and most urgent question to be answered is usually - is the spinal cord functionally transected, or is some neural activity being conducted through the damaged area? Until recently there has been very limited use of evoked potentials in evaluation of injuries of the nervous system and practically no application at all in the evaluation and monitoring of spinal cord injury. This kind of information is important not only in understanding the altered basic physiology of the nervous system, but in direct management of the injured patient.

In order to provide a more standardized approach to producing experimental lesions, an instrument has been developed that will deliver a calibrated injury force to the cord. This force can be displayed on an oscilloscope as a curve and photographed. In principle, a strain gauge is used to measure the force delivered to the cord.

Pharmacological studies at the Center have led to the finding that prostaglandin E_2 and prostaglandin F can be measured in spinal cord. Preliminary data suggest that prostaglandin E_2 levels are lower at the trauma site three days after trauma whereas the levels in the tissue are higher at the site of the trauma three hours after injury.

At another Center, laboratory studies of edema formation and blood flow in the area of injury have shown variations that are difficult to explain but appear to relate to the degree of injury and to the time interval following injury and may relate to factors effecting neural dysfunction. Studies of the changes in the level of norepinephrine in the injured segment and adjacent segments of the spinal column at varying intervals following injury and following the application of varying degrees of force have failed to confirm the hypothesis that norepinephrine has significance in the production of progressive central hemorrhagic necrosis. They have, however, led to an interesting observation that following spinal cord trauma there is an increase in tyrosine hydoxylase activity, the rate limiting step in catecholamine synthesis. This may explain some of the changes in the level of catecholamine associated with spinal cord trauma and the variation in the findings of different laboratories in normal and damaged spinal cord. Alterations in systemic blood pressure and secondary spinal cord flow patterns following trauma have been shown to protect the spinal cord against the hemorrhagic progressive central necrosis. Studies to find out whether these changes protect dysfunction in the white matter are under way. The ability of systemic hypotension to alter the pathological signs of progressive central hemorrhagic necrosis may explain some of the variations that were reported following pharmacological blocking of catecholamine production since all of these agents cause systemic hypotension. These findings have led to plans to investigate the mechanism responsible for the changes in the kinetic properties of the enzyme tyrosine hydroxylase in the traumatized area and to study further the alteration in capillary permeability and the regulation of blood flow to the injured spinal cord segment.

The development of a "pressure sore" in the spinal cord injured patient carries the risk of mortality from sepsis at worst but inevitably the prolonged and expensive morbidity of local wound care and often the need for surgical closure. Measures are being sought to prevent, modify or minimize the effects of such a complication. Open granulating wounds have been produced experimentally and the influence of enzymatic debridement, topical antibacterials, biologic dressings and systemic antibiotics are being evaluated.

Within the last year a Spinal Cord Intensive Care Unit has been completed at one of the Centers in a large metropolitan area. Activities there have been directed towards the solidification of the referral network in order to increase the number of patients available for study in the unit within the first few hours after injury. During a period of eight months a total of 28 patients ranging in age from 16 through 73 have been intensively investigated.

All patients were immediately randomized for medical treatment with epsilon aminocaproic acid for 10 days according to the research protocol. In addition all patients were treated with steroids. These drugs reduce the amount of hemmorhagic necrosis and edema following spinal cord injury.

Independent neurological evaluations were made on 25 patients. Examinations were performed daily for 14 days after onset of injury. A total of 444 independent observations were made by three examiners. Information as to the mode of treatment was withheld from the primary examiner to permit an unbiased approach and evaluation of the patient's status. These studies are continuing in order to provide statistically significant results which are not possible yet because of the small series of patients.

The main thrust of the laboratory investigations at this Center has been directed towards the subject of the free radical mechanisms in the degeneration of the spinal cord following trauma and the role of the biogenic amines in the degenerative and regenerative events following spinal cord injury.

At another Center spinal cord injury has been produced in animals, chiefly macaques, and the resulting systemic and local changes have been studied. The immediate results of significant cord injury are major shifts in blood volume and changes in the blood-spinal cord barrier. These changes have been demonstrated by fluorescent microscopy and electron microscopy to occur within minutes and have proven to be useful techniques to show the microvasculature in a functional as well as anatomic context. These major changes have been demonstrated in capillary endothelial and basement membranes, and are being correlated with other morphologic and biochemical parameters.

Base line data in the normal cord is being acquired and information about pathologic changes is beginning to come in. The most definitive conclusions are:

1. Blunt trauma at 300 g/cm does not eliminate blood flow in the white matter, although gray matter flow is severely impaired within 30 minutes.

2. Barrier changes as demonstrated by the fluorescent microscope and the EM occur within a very few minutes. Later progression is not fully documented, but the vascular changes progress to involve more and more of the cord.

3. There are major changes in cardiac output and circulatory blood volume after cord contusion.

These studies indicate significant damage to the microvasculature of the central gray matter occurs virtually at once. It cannot be said whether the damage is irreversible as of the moment of impact or whether these microvascular changes result in destructive events which are delayed for some hours and therefore might be amenable to treatment. However, the fact that the structural changes in vessels, while rapid, are progressive, suggests that pharmacologic or other measures might be found to arrest that progression.

Little work has been done to date to measure changes in regional blood flow resulting from acute spinal cord injury. Exact quantitative diagnosis of these changes could serve as a supplemental aid in prescribing the proper treatment for acute spinal injuries as well as assist in the development of understanding of the injury process itself.

In collaboration with the Department of Aeronautical and Astronautical Engineering the technique of hot-film anemometry has been applied to the measurement of blood flow in the region of the spinal cord. The hot-film anemometer is a device which incorporates a temperature sensing mechanism which records alterations in blood flow as a function of thermal change. It has the advantage of being a non-invasive atraumatic method of recording flow and is potentially suitable for human use. Preliminary results have indicated changes in the heat transfer coefficient associated with spinal cord injury.

With the hope of promoting a useful exchange of information which is expected to come out of the Acute Spinal Cord Injury Research Centers, the Institute recently initiated a series of workshops. At the first of these, in March 1974, a primary goal was to correlate and standardize the experimental model of spinal cord injury and to establish more efficient ways to collect, store, and retrieve data on experimental studies as well as from human patients.

The question arises as to whether these efforts are warranted unless a so-called major breakthrough occurs. The fact is, however, that minor degrees of preservation or restoration of neural function which can only be documented on a large series of patients would be of considerable significance in terms of both the functional capacity and longevity of patients.

MULTIPLE SCLEROSIS AND OTHER DEYMELINATING DISORDERS

Multiple Sclerosis (MS) affects approximately a half-million people in the United States and is considered to be one of the major neurological disorders. The onset of the disease occurs predominantly in the 20-40 year old group and is usually progressive for the remainder of the life span, although intermittent remissions lasting months or years are commonly experienced. The symptoms are quite variable, depending on the part of the central nervous system affected, but the general pathological findings are fairly constant, involving demyelinating of white matter and subsequent plaque formation. The etiology of the disorder is unknown, although many assume it to be either viral or immunological. This hypothesis is primarily based on information obtained from other demyelinating diseases. However, since myelin is a product of neuroglia cell, an aberration of glial metabolism is considered to be involved.

Still unexplained is the unusual geographical distribution of MS. The incidence increases significantly with latitude, the highest occurring in the north temperate zones of Europe and North America. Adult migrants to areas of relatively low incidence, such as Israel, retain the pattern of their native region, while children under 15 years of age assume the lower risks characteristic of natives of the country to which they have immigrated. The NINCDS through its Extramural Grants Program supports many research projects at University Medical Centers and a large number of private institutions or foundations dealing with varying facets of this disease. These studies are focused on understanding fundamental mechanisms of the disease.

Efforts toward accumulation of basic clinical data on MS have continued. Each patient in one clinic is classified as to type of MS, phase of illness and clinical rating of neurological disability (Kurtzke System) at each visit.

Sixty-six patients from the Los Angeles region have signed forms donating their bodies to the research effort. Two autopsies have been performed quickly after death. The specimens collected have been sent to investigators in Philadelphia, as well as at UCLA. The brains and spinal cords have been placed in the National Multiple Sclerosis Society Specimen Bank. Eight patients have been hospitalized for intensive study of blood, CSF and clinical state.

Another clinical study consists of a virological comparison of 144 patients with MS, 34 of their healthy siblings, and 40 patients with other neurologic diseases. Antibodies in serum and cerebrospinal fluid (CSF) to vaccinia and measles viruses were measured, and were correlated in the MS patients with the clinical characteristics of their disease. Cerebrospinal fluid antibody to vaccinia virus was both more frequent and at higher titer in MS patients than in either control group. Moreover, a significant increase was found in both frequency and titer of CSF vaccinia antibody in patients with the progressive form of the disease as compared with those classified as relapsing-remitting. Significant differences between MS patients and their siblings were not observed for CSF or serum measles virus antibody, although such groups had significantly higher serum antibody titers than patients with other neurologic diseases.

Delayed type hypersensitivity to killed vaccinia virus antigen was not different in MS patients and controls, although patients tended to have smaller vaccination scars than their healthy siblings.

The brains of several patients with MS have been examined in regard to vaccinia antigen, which can be identified either by fluorescent antibody or horseradish peroxidase labeled antibody to the various antigens of vaccinia virus. These studies continue to be inconclusive.

Another avenue of research is the isolation of infectious agents from tissue obtained from patients with MS and, when isolated, characterize and determine their relationship to the disease. One of the premier projects of one MS research center is to attempt the isolation of viral agents from autopsied MS brains. Its virology section has established more than 73 cell cultures from various portions of 12 brains from MS patients. In addition, control brain cultures were established from 8 cases of neurologic disorders other than MS, and 6 from non-neurologic cases.

Experiments with Tamiami virus were completed to provide a model for infection of the rodents central nervous system. This virus produces a clinical cerebellar ataxia and meningoencephalitis which, upon resolution, results in asymptomatic cerebellar heterotopia. It was learned that the entire clinical and pathological disorder was immune mediated, since the clinical disorder and pathologic changes were abolished by neonatal thymectomy. This model not only helps to provide a basis for further understanding of viral-induced immunopathology, but also provides a model for studying virus-induced anomalies acquired at birth and virus persistence in vivo.

Previous reports by one research group have described the isolation of a paramyxovirus from the brain cells of two MS patients. The agent referred to as the 6/94 virus has been studied extensively during the past year. It has been analyzed for its antigenic, RNA and polypeptide compositions and for selective biological properties in animals and cell cultures. Findings establish that 6/94 virus contains a 50S RNA genome and is related to Sendai virus in its antigenic and total polypeptide compositions.

Studies on the underlying mechanism for brain tissue destruction which is triggered by virus infection and persists in the absence of detectable virus is being continued. It is hoped that this system will provide clues for an elucidation of possibly virus-induced human encephalopathies such as MS.

Similarly, other investigators are currently completing studies of chronic infection with JHM virus and the role of the immune response in chronic demyelination and progressive multifocal leukoencephalopathy (PML). Some BALB/c mice have shown continuing demyelination 14 months following an intracerebral innoculation. Electron microscopic studies have shown the presence of coronavirus virions in oligodendrocytes. The frequency of this late process is currently being studied, and attempts to isolate virus both by conventional methods of homogenizing brain and innoculating into susceptible suckling mice and by explanting infected CNS tissue and co-cultivating with a susceptible cell line are in progress.

With respect to progressive multifocal leukoencephalopathy (PML), work on identifying the relevant viruses by electron microscopic agglutination and fluorescent antibody staining of frozen brain has been completed, indicating the JC virus was implicated in nearly every case. In addition, cells from the brain of a patient with PML were shown to be persistently infected with SV40. These cells contain the SV40 genome and modulate the expression of T antigen in a unique pattern with T present in dividing cells and disappearing as soon as contact inhibition occurs.

Studies of animals with chronic JHM infection have given further information on the role of virus in chronic demyelination and may help define the role of the immune response in this process. The detailed characterization of the papovaviruses associated with PML and studies of infected cells from the brain of a patient with PML have given new information on the agents associated with the only chronic human demyelinating disease demonstrated to be of viral etiology.

The ability of serum from EAE-affected animals to inhibit myelination has been examined at various periods during myelinogenesis. The data show that inhibition is more difficult to produce as myelinogenesis proceeds. In parallel studies EAE serum can inhibit cerebroside sulfate synthesis at advanced stages of myelinogenesis.

The possibility that CNS antigens other than myelin basic protein may be involved in the induction of allergic encephalomyelitis was supported by studies of sera from rabbits exposed to cerebroside in complete Freund's adjuvant. Their sera demyelinated and inhibited myelinogenesis in cultured tissues. Moreover, one rabbit developed clinical signs and histological lesions indicative of EAE.

The role of myelin basic protein in myelinogenesis was suggested but not conclusively proved by experiments in which it was added to the tissues' environment during myelinogenesis. Demyelinating factors appear in the serum of rabbits innoculated with basic protein or with encephalitogenic peptide. Chronic EAE has been produced in rabbits and guinea pigs, and their sera are under examination. A spectrofluorometric assay procedure to demonstrate antimyelin antibodies has been devised and is being applied to the study of EAE.

A vertical and a horizontal study of MS patients' sera are in progress. Previous ratios of active/inactive sera during exacerbations and remissions have been confirmed. Demyelination appears either to occur in parallel with exacerbations, with intervening periods of negativity during remissions, or else it is constantly present, sometimes in patients with chronic progressive forms of MS, sometimes in patients with no clinical evidence of disease.

EM studies of tissue exposed to human (MS) demyelinating serum reveal the characteristic pattern of demyelination, degeneration of oligodendroglia and, a new finding, an active engagement of astrocytes in phagocytosing myelin and degenerating oligodendroglia.

More detailed studies of measles virus and SSPE virus (5 different strains) have been performed. Cytopathic effects and the morphology of virus particles vary - suggesting that SSPE virus is a mutant of measles virus exerting a less deleterious effect on CNS tissue. The differences noted in the virus-CNS may account for the range of clinical disease patterns of SSPE in man.

A cell-associated (IP-3-CA) strain of SSPE virus has been examined. Budding virions were never observed, although infection spread throughout the CNS tissue. Also, no infectious virus could be demonstrated. The experiments suggest that a sub-viral genome or viral particle passes from cell to cell to cause the extensive CNS damage.

The 6/94 agent (parainfluenza virus rescued from an MS patient's CNS tissue) is also being studied. It replicates in hamster cerebellum in culture and produces a cytopathic effect which includes the disappearance of myelin. The uptake of herpes simplex virus by out-growing axons reveals the attachment of the virus particles, an invagination of the plasma membrane and its uptake into coated vesicles.

Marek's disease was induced in 200 chickens by exposure to chickens infected with JM strain of Marke's disease virus. Their sciatic nerves and nerve roots were studied by electron microscopy. Demyelination within perivascular mononuclear cell infiltrates was observed. Myelin sheaths were primarily damaged, leaving Schwann cells intact. Virus particles were not identified.

Experimental allergic neuritis (EAN) was induced in 20 chickens by peripheral nerve emulsions mixed with complete Freund's adjuvant. The lesions of EAN were compared to lesions in sciatic nerves of chickens of same age afflicted with Marek's disease (MD). The ultrastructural pathology of EAN and MD was identical.

Ten dogs were innoculated with Snyder Hill distemper virus. Acute distemper encephalitis, occurring about 10 days after innoculation, was studied by electron microscopy in 3 dogs. Virus was found in astrocytes and microglial cells, but not in oligodendrocytes.

C-type oncorna virus was innoculated in a variety of mouse strains, and the pathogenesis of a slowly developing myelopathy was studied by immunofluorescence and electron microscopy. Virus was abundant in neurons and oligodendrocytes causing neuronal degeneration and demyelination in the spinal cord in the absence of inflammatory cells. Virus invaded the CNS by budding from endothelial cells and spreading through extracellular spaces.

Studies on human adrenoleukodystrophy revealed pathognomonic intracytoplasmic inclusions in oligodendrocytes. Neurofibrillary tangles in cases of progressive supranuclear palsy were found to be composed of pathognomonic straight neurotubules of a diameter distinct from normal neurofibrils.

The pathogenesis of virus-induced post-infectious demyelinating encephalomyelitis and neuritis is not known. A morphologic approach is expected to show whether demyelination in these diseases occurs secondary to the destruction of virus infected myelin supporting cells, or whether demyelination follow a pattern identical to that seen in allergic encephalomyelitis and neuritis in which myelin sheaths are primarily damaged by an autoimmune reaction. The similarity of the experimental models to human post-infectious encephalomyeliti and neuritis suggests that the findings will contribute to the understanding of human demyelinating diseases.

Immunologic Markers in Developmental Brain Disease. An analysis of the genetic control of the immune response has been undertaken. Since other genetic sites beside the theta locus could have marked effects upon the influence of maternal factors in the development of the offspring's nervous and lymphoid systems, a more detailed analysis of the genetic control of T lymphocyte function was performed. Both T cells and brain contain the theta antigen; both systems undergo significant post-natal maturation. Development in this portion can be significantly altered by immunological activity of the mother. From this work a convenient and sensitive technique for measuring lymphocyte DNA synthesis was developed.

Demonstration of the Ir-EAE gene in rats has recently evolved from these studies and may have relevance to the pathophysiology of human demyelinating disease and its prevention. This is consistent with the hypothesis that susceptibility to EAE in rats is controlled by an autosomal dominant gene linked to the major histocompatibility locus. It was proposed that this is an immune response gene designated Ir-EAE, which controls T cell reactivity directed against the highly encephalitogenic portion of the basic protein molecule. The study indicated that the expression of the Ir-EAE gene in terms of clinical signs and severity of histological lesions may have been controlled by various other genes.

EAE is the best studied experimental model of an immunologically mediated brain disease. Since it is likely that both human MS and acute disseminated encephalomyelitis (ADEM) have an immunologic component in their mechanism, studies of the genetics of the immune response and how it may be regulated in EAE are pertinent to these common human diseases. Evidence that susceptibility to EAE is linked to the major histocompatibility locus in the rat is directly analagous to the observation that MS is correlated with HL-A-3 in the human and lends weight to the possibility that specific Ir genes in the human may be a significant determinant to the susceptibility of MS. Studies of genetic and immunological control of the expression of the Ir-EAE gene may provide models for analyzing the factors that control the severity of MS among susceptible individuals.

Work is in progress on the isolation sequence and modification of myelin proteins and the application of these products to the study of demyelinating disease. If some of these products are non-encephalitogenic but do invoke an immune response, it may be possible to apply them to the study of demyelinating diseases in humans. The purpose of this research has been to develop methods for the production of immune unresponsiveness in animals with EAE.

Previous studies have shown that EAE in the Lewis rat is a highly consistent and reproducible event, and this makes it particularly suitable, therefore, for this type study. It has been shown that guinea pig basic protein is highly encephalitogenic in the Lewis rat, whereas bovine basic protein has only mild activity. Lewis rat basic protein is intermediate. The same relative degrees of activity have been shown to exist for the 45 residue fragment from each of the 3 respective basic proteins. The synthetic tryptic peptide, T8-thr-thr-his-tyr-gly-ser-leu-pro-gln-lys which is moderately encephalitogenic in the rabbit, has no activity in the Lewis rat. There is reason to suspect that T8 is, nevertheless, part of the active site, and that the rat merely requires a longer sequence for full recognition. Attempts to synthesize a longer region around T8 which might be encephalitogenic in the rat have been unsuccessful to date.

It has been shown that EAE can be suppressed by the parenteral administration of basic protein either shortly before or at the onset of clinical signs. Attempts during the past year to induce immune unresponsiveness in the Lewis rat are not in accord with these findings. Bovine basic protein, however, has no effect under these circumstances, and rat basic protein is intermediate in its suppressive effects. The capacity of these 3 proteins to suppress EAE is consistent with their relative degrees of encephalitogenicity.

Preliminary studies suggest that induction of immuno-unresponsiveness by parenteral injections of basic protein can only be achieved with protein that possess encephalitogenic activity, although subtle modification of basic protein may achieve the ideal of protection without encephalitogenicity.

In other work, blood samples were obtained from 56 patients with MS, and 100 controls, and determinations made of histocompatibility types and of neutralizing (Nt) and hemaglutination-inhibiting (HI) antibodies to measles. A statistically increased frequency of HL-A type 3 was found in MS patients (42.9%). HL-A3 individuals showed higher measles antibody titers than non-HL-A3 individuals. The implication is that the increased measles antibody titers found by others in MS patients are related to the increased proportion of HL-A3 carriers. The relationship of histocompatibility type to immunological reactivity in general and to other viruses possibly relevant to MS is currently being investigated.

Studies of histocompatibility type and immunity to measles virus in MS may help to explain the heretofore perplexing finding of elevated measles antibody levels in this disease. It may also be possible to explain the peculiar geographic distribution of MS as a correlate of distribution of histocompatibility types in different populations. The histocompatibility type may also correlate with increased susceptibility to some other viral agent, possibly etiologic to MS.

It has been postulated that ingestion of cycad nut may be an etiological factor associated with the high incidence of amyotrophic lateral sclerosis (ALS) among the native Chamorros of Guam. Two toxic constituents, cycasin, an azoxy-glucoside, and the methylazoxy-methanol (MAM), the aglycone of cycasin have been isolated from the cycad nuts. Both compounds are hepatotoxic and carcinogenic. It has also been shown that MAM induces microencephaly in the offsprings of mother rats injected on the 15th day of pregnancy. There appears to be organizational changes at the intracellular level in this microencephaly, and these changes are being examined.

The long term goal is to produce with MAM neural degeneration and paralysis in experimental animals similar to ALS. The approach being used is to observe, analyze and compile as many biochemical and histological effects of cycasin or MAM on neural tissues as possible and to relate the changes to the time of exposure to the toxicant.

The long range objectives of one project are to isolate and study specific cell types and cell portions of the CNS and determine how each contributes to the chemistry and biochemistry of the whole tissue. Detailed examination of the sphingolipids of bovine oligodendroglia has been completed. Electron microscopic study indicates that astroglia are largely destroyed during this procedure.

Rats fed on a diet containing 500 parts per million (ppm) of hexachlorophene (HCP) developed cerebral edema and spongy changes in the white matter. Microscopy showed that the lesions consisted of vacuoles between lamellar in the myelin of the brain and later work demonstrated that myelin in the CNS and PNS was split at the intraperiod line. HCP myelinopathy may be a model system for demyelinating disease and to try to correlate biochemical changes in myelin with morphological evidence of damage to myelin. The morphological changes are similar to those seen in triethyltin edema. In that model myelin is considerably decreased, and its composition is altered in a non-specific way (lowered cerebroside, elevated cholesterol).

Other work is concerned with elucidating the processes which maintain the stability of myelin. Proteolipid protein constitutes the major myelin protein. The study of its properties, its amino acid sequence and its interactions with other brain components is, therefore, pertinent to understanding the structure of myelin. Preliminary observations suggest the possibility of a role for proteolipids in a slowly developing demyelinating process. This may provide a closer analogy to MS than does the acute experimental allergic encephalomyelitis model.

An investigation of the biochemistry of the special lipoproteins designated as proteolipids (PL) and of their proteolipid apoprotein (PLA) has established that these characteristically water-insoluble chloroform-methanol (CM) soluble lipoproteins consist of an apoprotein, which is combined with different lipids. Study of the brain central white matter proteolipids and their apoprotein had shown that the truly characteristic part of the proteolipids is the apoprotein, to which various acidic lipids may be combined by ionic linkages, and with which most lipids and many other substances may form complexes.

In addition, there is an attempt to derive a comprehensive model which will embody a total working hypothesis for constitutional, diffraction and charge distribution phenomena. Though there may be some slight differences in the periods within the CNS and PNS groups, the more significant difference of ca. 160 Å (CNS) to the average 184 Å of PNS myelin remains, without transitional examples within this gap. The most obvious explanation is that the myelin-generating satellite cells - Schwann Cells in PNS and oligodendrocytes in CNS - by introducing differing components (mostly protein differences in all probability) determine the variations in structural period along with contrasts in swelling abilities and the related membrane charge distributions.

Investigators have attempted to examine the changes in the activity of myelin lipid hydrolytic enzymes in sciatic nerve undergoing demyelination after crushing injury, and studies have been extended to include human peripheral nerve biopsies from subjects with diseases of demyelination or arrested myelination. Results have shown that levels of B- galactocerebrosidase activity in rat brain homogenates were similar to those obtained by other investigators using the same incubation medium. This experiment also revealed detectable activity in rat sciatic nerve. This is important because it allows the study of enzyme activity in small amounts of peripheral nerve such as human sural nerve biopsies.

Subjects with hypertrophic neuropathy and their families have been identified and the lipid class composition analyzed on sural nerve biopsies. The lipid composition of the sural nerve in these subjects was characteristic of 1-2 years of age in contrast to the previous case, which had sural nerve lipids characteristic of 24 fetal weeks. Minimal loss of myelin lipids was detected in sural nerves of other family members only when conduction velocity was prolonged.

The assay of galactocerebroside B-galactoside activity in human peripheral nerve biopsy material may help to establish whether the deficiency of peripheral nerve myelin lipids in Dejerine-Sottas disease is due to demyelination or to arrested myelination. The significance of these studies is an approach to arrest the process of demyelination at a molecular level. Regardless of the initial causative agent, the changes in membrane lipids are similar. Certain common enzymes involved in the normal degradation of myelin components are either hyperactive, or the lysosome which contains these enzymes releases them excessively.

Investigators using computational technology have developed electrophysiological means of assessing the neurological status of MS patients and patients with spinal cord injury, understanding the nature of symptom production in MS and the development of methods of therapy for MS patients in the absence of known etiology.

Integration of this system into the MS laboratory is being accomplished through a central signal conditioning rack. Application software is being developed to support the MS studies. In particular, a Fast Fourier Transform has been implemented. These studies will provide insight into the mechanisms of symptom production as well as the natural course of MS. The experimental techniques being implemented have proven to be sensitive measures of impaired neuronal conduction. This work will also be applied to improving methods of assessing neuronal conduction in patients with MS. These techniques should be of great importance in any future drug studies or in the evaluation of any other form of treatment in this disease.

PARKINSON'S DISEASE

Parkinson's disease is a progressive neurological disorder of unknown cause affecting certain brain areas responsible for the control and regulation of movement. Estimates of prevalence range from one case per 1,000 to one per 200. Primarily a disorder of middle age or later, its prevalence is thought to be increasing with the increase in the average life span.

The onset of parkinsonism is ultimately characterized by tremor, rigidity, and bent posture. Intelligence is usually unaffected. In the fully developed disorder the face becomes the characteristic "mask" with a hard stare coming from unblinking eyes. The patient often sits motionless, rarely crossing his legs or folding his arms.

Standard treatment in the past consisted of physical therapy, a variety of drugs, and surgery. The treatments of choice are now thalamic surgery or a highly effective form of replacement therapy making use of large oral doses of L-Dopa. Although L-Dopa induces remarkable improvement in most parkinsonian patients, it has very unpleasant side effects and some patients do not respond. Therefore, a still better treatment must be found and the greatest long range contribution of the research on L-Dopa may be that, as the first direct lead to the real cause of parkinsonism, it provides an approach to the development of a much more effective form of therapy. Despite many recent developments, evidence indicates that the newer methods of therapy have not substantially increased life expectancy.

Recently numerous biochemical abnormalities in Parkinson's disease have been found. Most of these findings are related to a decrease in dopamine concentration in the parkinsonian brain. Most of the biochemical changes in dopamine metabolism in the parkinsonian brain can be duplicated by making a lesion in the substantia nigra in animals. The changes in acetylcholine metabolism in Parkinson's disease, reflected by the hypersensitivity to cholinergic stimulation and therapeutic improvement with anticholinergic drugs, also are secondary to the loss of nigro-striatal neurons. The degeneration of the substantia nigra cells and certain other neurons appears to be the primary changes in this disease. Most investigators agree that it is only the neurons which contain melanin (e.g. substantia nigra, locus coeruleus, dorsal nucleus of the vagus, etc.) which degenerate in Parkinson's disease. This suggests that there is some peculiarity of the neuromelanin-containing cells of the central nervous system which predispose them to degeneration in parkinsonism. Investigation of the structure, mode of synthesis and possible function of neuromelanin is one important approach to the etiology and pathogenesis of idiopathic Parkinson's disease. Attempts are being made to determine if there is any biochemical inter-relationship between substantia nigra melanization and the abnormalities in catecholamine metabolism in this disorder.

The aim of one research program is to develop an understanding and prevention of such unwanted effects of L-Dopa therapy as drug-induced psychotic behavior and involuntary movements. The hope is to separate these actions of L-Dopa from the therapeutic action against Parkinson's disease. It is also hoped that these studies and similar ones to be performed on psychotic patients will provide new forms of therapy for naturally occurring psychoses as well as those induced by L-Dopa.

The research focus is to attempt to control psychotic behavior and dyskinesia with cholinergic drugs such as physostigmine, oxotremorine or piperidine while at the same time not interfering with the control of Parkinsonian symptoms by L-Dopa. It is felt that the hypothesis that Parkinson's disease results from a loss of inhibition of a striatal cholinergic neuron by a nigrostriatal dopaminergic neuron is overly simple and the work will be continued without such a hypothetical model.

The preclinical work includes toxicological studies of the agents used and tests of the agents on various animal models. These models include modification of the ability of L-Dopa to induce turning behavior (toward the side of the lesion) in caudectomized mice and actions of cholinergic drugs on caudectomized mice. Since cholinergic drugs modify the behavior of mice intoxicated by L-Dopa, other intoxications will also be studied such as those of LSD and amphetamine.

Clinical studies to date have tested only physostigmine and it has been established that relatively large doses of this compound can be tolerated by Parkinson's patients, provided that dosage is increased gradually and probanthine is given. The initial results on four patients have been promising. Garrulity and hyperactivity of patients on L-Dopa has been decreased while affecting the control of parkinsonian symptoms relatively little. No experience has yet been gained with the treatment of natural psychoses.

Clinically, attempts are being made to control troublesome side effects of L-Dopa which greatly limit its therapeutic effectiveness. If these side effects can be alleviated without producing significant deterioration of the therapeutic action on parkinsonism, it will be of great clinical benefit. Improvement with the administration of physostigmine to those with mental abnormalities secondary to L-Dopa has been demonstrated. Other investigators have demonstrated the effectiveness of physostigmine in treating toxic psychoses and in decreasing the involuntary movements of Huntington's chorea. Anticholinergic drugs do not produce abnormal involuntary movements or increase those produced by L-Dopa. However, although not identical, there is some similarity between the mental effects of L-Dopa and those of anticholinergic toxicity, and the latter can potentiate the former. Thus, there is a certain rationale from clinical experience for the use of cholinergic agents to alleviate both the mental effects and the abnormal involuntary movements produced by L-Dopa. Fluctuations in the therapeutic response constitute another troublesome aspect of therapy with L-Dopa. Preliminary studies on the effect of low protein diets plus MK 486 in smoothing out this response are very encouraging.

Effects of apomorphine and related compounds are being investigated. The complex neurological effects of apomorphine are viewed as resulting from three somewhat overlapping parts of the molecule, viz. a 3, 4-dihydroxyphenylethylamine moiety that produces jumping and garrulity similar to that from levodopa, a phenylethylamine moiety that leads to head shaking, and a 1-methylpiperidine moiety that yields sedation. In support of this hypothesis it has been observed in nialamid-treated mice that the combination of equimolar doses of dopamine, phenylethylamine and piperdine led to behavior indistinguishable from that from apomorphine. New compounds are being synthesized to try to incorporate the desirable portions of the molecule. In addition, work is also being extended to include a tropolone moiety in order to add inhibition of COMT to the properties of the compound. The ideal substance would inhibit COMT and peripheral AAD. Toxicity and behavior of new compounds will be assessed in mice before these drugs are given to humans. N-propylnorapomorphine, a drug now commercially available and said to be 50 times more potent than brain apomorphine as a dopaminergic agent, will be administered to Parkinsonian patients in an attempt to obtain the beneficial effects of apomorphine without the production of prerenal azotemia. The drug will be given orally in gradually increasing doses, a technique that circumvented nausea and vomitting in earlier studies on apomorphine. Renal function will be kept under close scrutiny. If the drug emerges as a therapeutic option, it will be studied in conjunction with growth hormones and a variety of dietary factors.

Relationship of manganese to L-Dopa is also being investigated. This group of studies proposes to show a relationship between the metabolism of manganese and that of biogenic amines by studying (1) biopsies of various regions of the brain of cats which had received 100 to 400 mg/kg of L-Dopa I.P. Studies of this sort would form the basis for future tests on human beings. It would be desirable to have a measure of the amount of dopa entering the brain of Parkinsonian patients following dopa therapy with the hope that the use of tracer doses of radioactive manganese can be used for this purpose; (2) the effects of dietary manganese intake; (3) the effects of prednisolone; and (4) the effects on the fetus of dopa administered to the mother. Manganese will be determined by neutron activation analysis by the "non-destructive method" and then dopa and dopamine will be assayed. If a link can be established between manganese and dopa, this research would be a worthwhile contribution to the understanding of the pathogenesis of Parkinson's disease and possibly give a clue concerning the binding, storage, or mechanism of action in the CNS.

Studies by another investigator are aimed at correlating biochemical (biogenic amines) parameters with neurological abnormalities associated with extrapyramidal disorders. In these studies monkeys with surgically induced lesions in specific brain areas who exhibit abnormal movements are used as an experimental model. The effect of the lesions on putative neurotransmitters is being evaluated. Histological changes resulting from the destruction of various anatomic pathways are to be correlated with biochemical changes. The clinical studies involve an evaluation of the effectiveness of L-Dopa therapy and an investigation of the fate of L-Dopa in patients undergoing treatment for extrapyramidal disorders.

Development of a primate model of parkinsonism, producing tremor and hypokinesis, has been an important feature of this project. The administration of L-Dopa in combination with 1-alpha-methyl-dopa hydrazine (MK-486), a dopadecarboxylase inhibitor that acts peripherally, resulted in a transient disappearance of tremor in the extremities contralateral to the lesion with a concomitant development of involuntary movement. Trivastal (ET-495), an agent that stimulates dopamine receptors, had an effect similar to that of L-Dopa. Trivastal relieved the tremor and induced involuntary movements for a longer time than L-Dopa. 2-Br-alpha-ergocryptine (CB 154), another agent that stimulates dopamine receptors, also has tremor relieving and involuntary movement inducing activities.

The effects of activation and blockade of dopamine receptors on dopamine synthesis in striatal slices show that apomorphine, an agent that stimulates dopamine receptors, inhibits the biosynthesis of ^{14}C-dopamine from ^{14}C-tyrosine more effectively in striatal slices than tyrosine hydroxylase activity _in vitro_. The effective inhibition of ^{14}C-dopamine biosynthesis in striatal slices by apomorphine could either be due to its accumulation in the dopamine containing neurons and subsequent inhibition of tyrosine hydroxylase or to dopamine receptor stimulating activity of the drug resulting in a feedback control of dopamine biosynthesis. In order to study further the effects of activation or blockade of dopamine receptors on presynaptic dopamine synthesis, the effects of Trivastal and of Haloperiodol on ^{14}C-dopamine synthesis from ^{14}C-tyrosine in striatal slices is being investigated. Trivastal stimulates dopamine receptors, but unlike apomorphine, does not contain a catechol group and does not inhibit tyrosine hydroxylase activity _in vitro_. The administration of Trivastal results in an inhibition while administration of Haloperidol results in the stimualtion of ^{14}C-dopamine synthesis in striatal slices. Treatment of rats with Haloperidol antagonizes the Trivastal induced inhibition of ^{14}C-dopamine synthesis. These results support the idea that a receptor mediated feedback exists which controls the rate of dopamine synthesis.

The effects of dopamine agonists on tremor in monkeys with ventromedial tegmental (VMT) lesions were investigated. The finding that some drugs which inhibit prolactin secretion have the property of stimulating dopamine receptors was fundamental in screening for new antiparkinsonian agents. Trivastal, 2-Br-a-ergocryptine (CB-154) and Lergotrile inhibit prolactin secretion and all three compounds presumably stimulate dopamine receptors. These compounds, therefore, were tested for their antiparkinsonian efficacy in monkeys with VMT lesions. Subsequently clinical studies have been initiated in parkinsonian patients.

Trivastal: The single administration of Trivastal resulted in a dose-dependent relief of the spontaneous tremor in monkeys with VMT lesions. At a dose of 3 mg/kg (i.v.) the tremor disappeared for 3-4 hours and the intensity of the tremor was diminished for an additional 2 hours. The administration of Trivastal resulted also in the development of involuntary movements. These results indicate that tremor and involuntary movements are associated with a common mechanism and that the activity of the dopamine receptors are involved in the regulation of these dysfunctions.

Lergotrile: The single administration of Lergotrile resulted in a dose-dependent relief of the spontaneous tremor in monkeys with VMT lesions. Following a single administration of Lergotrile at a dose of 5 mg/kh (i.p.) the tremor disappeared for 1-2 hours and the intensity of the tremor was diminished for 7-9 hours. Repeated administration of Lergotrile reduced its anti-tremor effectiveness. Following the third administration of Lergotrile (third day of treatment) the spontaneous tremor was only relieved for 3-5 hours but did not completely disappear. Sedation and compulsive circling behavior towards the denervated side, but no chorea-type movements, were observed in all monkeys following a single administration of Lergotrile. No sedation or chorea-type movements were observed following repeated administration of the drug.

CB-154: The administration of CB-154 (6 mg/kg, i.p.) resulted in the disappearance of the tremor for 1-27 hours. Repeated administration of CB-154 (5-8 mg/kg, i.p.) resulted in the disappearance of the tremor for at least 48 hours. After cessation of treatment the intensity of the tremor was diminished for another 24-48 hours. The repeated administration of CB-154 resulted in the development of involuntary movements. However, the involuntary movements were of moderate intensity and of shorter duration than the drug-induced relief of the tremor. Only one of the monkeys with spontaneous tremor developed unilateral chorea-like movements following repeated administration of the drug. The data suggest that CB-154 could be a powerful anti-tremor drug in parkinsonian patients with unusually long-lasting actions. In this respect it has considerable advantages over dopa and ET-495, although the latter compound also possesses relatively long-lasting actions. Furthermore, the involuntary movements are only of moderate intensity after CB-154 and have not been seen for more than a day, whereas the anti-tremor activity can last for 4-5 days.

In clinical studies thirteen patients with Parkinson disease were treated with Lergotrile and an overall improvement was observed in some patients. Tremor appeared to be the main clinical feature to benefit and reached statistical significance. In a subgroup of four patients treatment with a higher dose of Lergotrile resulted in further improvement in all cardinal signs but only the improvement in tremor was statistically significant. Adverse effects included orthostatic hypotension, behavioral alterations and gastrointestinal disturbances, but were severe enough to require drug withdrawal in only one patient. Involuntary movements that could be related to Lergotrile were not observed.

Another laboratory has found that L-Dihydroxyphenylalanine (L-Dopa), administered in high doses, produces bizarre alterations in human and in animal behaviors. Reported side effects of L-Dopa therapy include: insomnia, depression, hypomania and paranoid reactions. Alterations in animal behavior include psychomotor excitement, aggression, sterotyped behavior and hypersexuality. The aim of this research is to more clearly define the neurochemical mechanism of these behavioral changes induced by excessive brain Dopa concentrations, in an attempt to understand the behavioral side effects of L-Dopa therapy in human disease.

L-Dopa produces increased dopamine levels in the brain but it also may acutely release serotonin and then inhibit its synthesis. Many of the behavioral alterations produced by Dopa may be due to functional or metabolic alterations in serotonergic neurons. The main emphasis of the proposed research will be to study this relationship. Drugs which block serotonergic receptors (Methysergid, 2-bromo-LSD, and cyproheptadine) or deplete serotonin (p-chlorophenylalanine, p-CPA) will be used as pharmacological tools to study the role of serotonin in the effects of L-Dopa. Reduction of certain L-Dopa behavioral effects by these compounds would indicate indirect (non-dopamine) mediated behavior (locomotor activity depression acutely and enhancement when 5-HT is depleted). Behavioral analyses would be complemented by biochemical analyses of L-Dopa's influence on serotonin, dopamine and norepinephrine turnover rates and concentrations in specific brain areas.

The long-term goal of the proposed research is to determine the neurochemical mechanisms through which non-physiological body loads of the catecholamine precursor L-Dopa produce biphasic actions on certain animal behaviors. The specific objectives include (1) determination of the mechanism(s) and site(s) by which low doses of L-Dopa (320 mg/kg i.p.) reduce spontaneous locomotor activity of male mice; (2) determination of the mechanism(s) and site(s) by which high doses of L-Dopa (560 mg/kg i.p.) enhance spontaneous locomotor activity; (3) studies to determine if tolerance develops to either or both of the above effects of L-Dopa and elucidation of neurochemical mechanisms by which such a tolerance would be produced. While the basic behavioral measurement to be made in this research is spontaneous locomotor activity in male mice, it is felt that useful generalizations about the mechanisms and sites of action of L-Dopa can be drawn only if these hypothesis are tested with a few other well defined behaviors. The decision as to which additional behaviors might be most appropriate to study will be made only when experiments designed to provide evidence regarding the three specific objectives have been substantially completed. At this time, preliminary evidence suggests that "Sidman Avoidance" (a behavior in which the male mouse has an extremely high response rate) and "platform" passive avoidance (characterized by lack of response) may be the most useful behavioral measurement to correlate with measurements of spontaneous locomotor activity.

The value of levodopa in the therapy of Parkinsonism is related to its decarboxylation to dopamine in the extrapyramidal brain tissue. It is generally believed that patients with Parkinson's disease have a lower than normal brain concentration of dopamine. Specific transport systems appear to be involved in regulating the access of exogenous levodopa to the sites of dopamine synthesis. L-Dopa transport in the brain has been extensively studied _in vitro_ but much less is known about its fate _in vivo_. The active transport of this compound in guinea pig brain slices has been demonstrated by a system shared by other aromatic amino acids but not by glutamic acid or alanine. In contrast, following an intravenous injection of 14C-L-Dopa in rats, the brain-to-blood concentration ratio was very low compared with other tissues such as liver and kidney. However, it has also been shown that L-Dopa is rapidly metabolized after intravenous administration with maximal concentrations of metabolites appearing in the blood after only 10 minutes. Thus, accurate information on the _in vivo_ cerebral transport of L-Dopa is difficult to obtain using conventional procedures. Recently, a ventriculocisternal perfusion technique has been successfully used to study the clearance of many types of compounds from the CSF. This method eliminates the necessity of intravascular injection and, therefore, reduces the problem of rapid metabolism.

The work proposed here is aimed at further clarification of the factors influencing the concentration of levodopa at its site of action and in the plasma. Previous studies on the renal transport of amino acids have demonstrated a high degree of structural specificity in the tubular reabsorption of L-tryptophan and a bidirectional transport system for the aromatic amino acids. In the proposed studies on levodopa transport, the ventriculocisternal perfusion and renal microperfusion techniques will be employed. The major objectives are to determine (1) the characteristics of efflux of levodopa and related compounds from the CNS using clearance from the cerebral ventricles as a model system; (2) the mechanism of renal excretion of levodopa and related compounds; (3) the effects of structurally related compounds and metabolic inhibitors on the CNS and renal transport of levodopa.

The large patient population of Parkinsonism and allied diseases accumulated over the past several years by one laboratory continues to be documented and studied with emphasis on the following: defining the effects of pharmacological agents on the natural history of the disease; testing the therapeutic efficiency of newly developed agents; monitoring side effects and toxic reactions of presently employed treatment programs; and searching for new insights as to the etiogenesis and pathogenesis of these disorders. Central to this program are the developments regarding the effects of monamines, particularly dopamine, on the symptoms of basal ganglia disorders. These investigators have been deeply involved in the application of these new findings from a diagnostic and therapeutic viewpoint as well as their implications as to the causation of these disorders. Essentially this program is continuing documentation of cases of Parkinson's disease and other types of parkinsonism. In accord with a specially designed protocol, including a rating scale for Parkinson signs and symptoms, patients are evaluated at regular intervals. These data have been useful in delineating the natural evolution of the Parkinson process and determining the effects of various treatment programs. This patient population also acts as a resource for exploring biochemical abnormalities in CSF and other body fluids which may exist in this disorder. In this regard, emphasis at present is on the various monamines, their metabolites and enzymes as well as the essential amino acids. Additionally, autopsy material is periodically being derived and correlative clinical and pathological studies carried out. The long term assessment of the therapeutic effectiveness of levodopa in parkinsonism is being investigated. The large patient population under treatment will continue to be examined at regular intervals as to the state of their parkinsonism, evidence of side reactions and/or toxic effects from the use of this agent. Here again, specific studies to identify metabolites of levodopa in CSF are in progress with a view to determining correlations with beneficial as well as side effects. When available, autopsy material is obtained and studied to determine the effects of administration of levodopa on the pathological Parkinson process in brain as well as its effects on other organs.

In general the therapeutic effectiveness of levodopa has continued to be superior to that of other therapeutic measures. It is, however, becoming increasingly apparent that it does not halt the progression of the disease and that after long-term usage, less uniform therapeutic response occurs and a greater incidence of CNS side effects develop. The two major side reactions limiting the clinical usefulness of levodopa are induced abnormal involuntary movements and a diurnal variation in therapeutic effects, the so-called "on-off" response. Special studies as to the incidence, nature and measures for control of these reactions are being carried out.

The studies so far indicate that 70-80% of patients on long term levodopa therapy develop abnormal involuntary movements in varying degrees. They tend to make their appearance in that segment of the body first involved in the Parkinson process and are dose and time dependent. In patients coming to autopsy in whom such movements have existed, no morphological changes were found. Except for reduction of the daily dose of levodopa, no effective means for their control have been found. Over the past years clinical trials have been conducted with the following: L-trytophan, administered in doses of 2-4 gms a day, has failed to alter the movements; similar negative results have been obtained with 5HTP. A trial of N-Butyl-gallate-alpha-methyl-transferase inhibitor did not alter these adventitious movements nor were they effectively diminished by the use of 3-0-methyl dopa. In an attempt to find clues as to the possible mechanism of these movements, extensive studies were carried out of the amino acid content of CSF in patients exhibiting this phenomena. In preliminary studies it was found that the free amino acid content of basal ganglia disorders is elevated. This is particularly true for leucine, valine and arginine. After prolonged levodopa administration and with the appearance of abnormal movements, these abnormalities are increased. The significance and implications of these abnormalities are being further explored.

The on-off effect reaction, which appears to be a complex form of an akinetic-rigid state, in its "off period" is intimately related to the use of levodopa. It bears a relationship to the time intervals of treatment in that in most instances it has made its appearance after months of treatment with levodopa. It occurs in some 15-20% of patients and its causation is unknown. Two patients were monitored for blood levels of dopa over periods of 8-10 hours and found marked alterations which can be correlated with the "on" and "off" periods. Both patients were unable to sustain a consistent uniform level of dopa and the off periods occurred when their blood levels of dopa dropped below a critical level of 5 um/l. Attempts to sustain a consistent blood level alone, by increasing the daily dosage or shortening the dosage schedule, resulted in less frequent "off" periods but an intolerable degree of involuntary movements. Alternate means of controlling this phenomena by lowering daily protein intake and using other anti-parkinson agents has to date been less than satisfactory. Further studies of this perplexing phenomena are contemplated.

A number of clinical trials involving new anti-parkinson agents are being carried out now. Twenty-two patients were treated with benopryozine-HCL dosages varying from 200-300 mg/day. Effective antiparkinson action was obtained with relatively few side effects. The drug is well tolerated with levodopa and potentially may prove valuable as an ancillary agent. A larger scale drug trial is planned.

N(5-pyrrolidino -3-pentynyl) succinimide is an analogue of oxotremorine which effectively blocks pharmacological action in laboratory animals. In preliminary trials carried out in 6 patients, a rapid effect in reducing tremor akinesia and rigidity in parkinson patients was observed. However, a number of side effects including dizziness, nausea and behavioral disturbances occurred. These latter may be related to dosages administered in these preliminary trials. A trial beginning with a more limited dosage schedule and an attempt to use this agent with levodopa will be carried out in the future.

DL serine N^2-(2-3-3-trihydroxybenzl)-hydrazine hydrochloride is a dopa decarboxylase inhibitor whose action occurs primarily in extra cerebral structures. When given in combination with levodopa it can substantially reduce the required dosage and reduce the incidence of side effects originating in peripheral structures. It has been administered to 6 patients who had previously received levodopa in maximal dosage, who had less than an optimal response, and who were experiencing side effects. In all patients it was possible to effectively reduce the required dose of levodopa by 70-80%. A therapeutic ratio of levodopa to the inhibitor of 4 parts to 1 was found most efficacious. However, the combination failed to fully obviate central side effects which some of these patients were experiencing. More intensive study of this agent is needed to fully establish its value in the treatment of parkinsonism.

Alpha methyl dopa hydrazine is also capable of peripheral dopa decarboxylase inhibition. In trials to determine its therapeutic value in parkinsonism to date it has been administered to 184 patients. It effectively reduces the required daily dosage of dopa, shortens the induction phase for dopa, and reduces the incidence of side effects such as nausea, vomiting and anorexia. To date, no side effects attributable to this hydrazine compound have been encountered. The therapeutic response is obtainable in most patients with a ratio of 10 parts of levodopa to 1 part of alpha methyl hydrazine. On the average, patients require 1.0 gm of levodopa and 100 mg of alpha methyl hydrazine which can be divided into 4 equal doses.

Following the initial report that levodopa was primarily methylated to 3-0-methyl dopa compound which was then capable of being converted back to dopa and dopamine, trials of its effectiveness in parkinsonism were initiated. In 8 patients treated with 3-0-methyl dopa, up to 6 gms a day, no alteration in parkinson symptoms were obtained. Additionally, CSF studies failed to show a rise in dopa following its administration though 3-0-methyl dopa rose to considerabel levels. It has, therefore, been concluded that this agent is ineffective in the treatment of parkinsonism.

The search for a viral agent in Parkinson's disease, especially the post-encephalitic form, is receiving increased attention. Recent observations demonstrated influenza virus antigen in brain sections of 6 patients with post-encephalitic parkinsonism by direct immunofluorescence staining. Specific immunofluorescence was seen in nuclei of cells in the substantia nigra and hypothalamus, and was apparently strain-specific for the neurotropic strains of type A influenza virus. Such fluorescence was not seen in brain sections of 6 patients with idopathic Parkinson's disease. These findings constitute the first important laboratory evidence, so far, that certain strains of influenza A virus may be linked with post-encephalitis Parkinson's, and that the latter is probably etiologically different from idiopathic Parkinson's disease. This study, however, does not furnish absolute identification of the viral agent, nor does it presume to offer conclusive evidence for causal relationship. Moreover, these findings are difficult to fit with presently known clinical and epidemiological data, the postulated relationship of encephalitis lethargica, post-encephalitic Parkinson's and influenza having become less tenable than it had been in the 1920's. The brains of a well-documented case of post encephalitic Parkinson's and a case of Parkinson's disease with dementia are now being studied.

There are two major routes of dopa metabolism. The first involves decarboxylation to dopamine and subsequent catabolism of dopamine to O-methylated, deaminated, and beta-hydroxylated products. The second major route of catabolism involves transamination processes to pyruvic and lactic acid derivatives.

It has been suggested by one investigator that the prolonged time course of the therapeutic effect of L-dopa results from the accumulation of one of its metabolites, 3-O-methyl dopa, which is slowly de-O-methylated to dopa and subsequently decarboxylated to dopamine. A correlation between neurologic status, psychiatric status, and metabolism of L-dopa is being sought. A population of patients with parkinsonism are placed on a standardized regimen of therapy with L-dopa. They are evaluated in great detail with regard to their neurologic status, psychiatric status and levels of urinary dopamine and dopamine metabolites in the drug-free state. The major finding thus far in this study of 16 patients with primary parkinsonism is that the pretreatment levels of each of the two metabolites of dopamine, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), are specifically correlated with the severity of the neurologic disease or with a specific indication of mental status. DOPAC varies with the severity of the neurologic disease, and HVA, with the psychiatric status. The primary objective of this study is to confirm these observations and to extend the findings to patients other than those with primary parkinsonism. Additional correlations will be examined between the metabolites and the therapeutic, as well as the adverse effects of the drug in an attempt to clarify the numerous problems and to lend additional data to evaluate the several theories which have been put forth to explain the clinical observations.

A strong relationship between the sulfate conjugation of a major metabolite of L-Dopa (3,4-dihydroxyphenylacetic acid, DOPAC) and the severity of parkinsonism is suggested. It has been found that administration of large amounts of L-Dopa to patients results in saturation of the mechanism for sulfate conjugation of L-Dopa metabolites. It was, therefore, of considerable interest to explore the biochemical determinants of the sulfurylation process. The experimental approach used here is a comparative study of the sulfate-conjugating systems in the liver and brain which may participate in the metabolism of L-Dopa or its metabolites in the intact mammal. The program is directed to determine the relative capacities of the liver and brain to generate sulfate esters of L-Dopa and its metabolites, and to examine the ability of dopa metabolites to saturate these systems. Also, it will compare the enzyme systems of the brain and liver, which generate the sulfate conjugates of L-Dopa and/or its metabolites, with those reported to catalyze the production of sulfate derivatives of other substances (e.g., phenol, serotonin, estrone).

Small concentric hyaline spherules are a distinctive feature of the form of parkinsonism known as paralysis agitans. These round structures, known as Lewy bodies, occur in various pigmented neurons throughout the brain stem (e.g., substantia nigra and locus coeruleus). They occur most frequently in idiopathic paralysis agitans, less frequently in the postencephalitic variety, and small numbers of them are also encountered in 5-20% of "control" autopsies. When examined by electron microscopy, the bodies are composed of filamentous structures which are more densely packed in the central portion of the spherule. The precise biochemical nature of Lewy bodies is not known. Efforts are being made to collect these bodies in greater number for chemical and morphological analysis. The answer would be of interest in relationship to the problem of parkinsonism, even though the specific nature of this relationship is not evident at the present time.

VIRUSES AND NEUROLOGICAL DISORDERS

Earlier workers in virology suspected the possibility that some viruses might remain dormant in the body for a prolonged period before producing overt disease and that others may be responsible for presistent and/or recurrent infection. Most of the clinical and experimental viruses are identifiable by a relatively short incubation period lasting only days or at the most a few weeks, with rapid proliferation of virus prior to the acute phase of disease followed by the disappearance of virus resulting in an increase in specific antibody that is supposed to provide protection against future infection with the same virus. Opposed to rapid onset of viral disease, virus infections with a prolonged incubation period and slow initiation of pathological processes may be responsible for many chronic and degenerative neurological disorders of man. Added evidence to the concept of "slow viruses" was gained when veterinary virologist Sigurdsson identified three infectious diseases that affected native Icelandic sheep not observed anywhere else previously. Two were neurologic and one a pulmonary disease. All had a protracted incubation period which resulted in the animals' death. Since then several other slow viruses both in animals and man have been found which specifically attack the nervous system. This report in general is related to these neurotropic viruses.

It is generally agreed that latent agents are those which can be detected more or less regularly by available techniques and which can persist in the organs of animals in the absence of symptoms. Masked (occult, hidden) agents, on the other hand, are those which are suspected as being present but which cannot be demonstrated directly by available techniques. The present distinction between masked and latent viruses, therefore, is conditional, and is dependent upon the sensitivity of available detection techniques. This leads to confusion, because some viruses now considered "masked" may be fully infectious, but be present in such small quantities or in such a physical state that present methods are inadequate for their demonstration. If an effective detection technique should become available or if existing techniques should be made more sensitive, then these viruses would be detected and therefore designated as "latent."

It is apparent, therefore, that there are two prime considerations in evaluating viral masking latency (1) the methods available for detection, with special reference to their sensitivity; (2) the basic nature of the virus-host relationship. The two considerations can hardly be separated in studies of masked and latent viruses. Although some insight has been obtained concerning the possible mechanisms of infectiveness in certain viruses, the approaches to unmasking "hidden" (or "occult") animal viruses have been largely empirical. Little is really known about why certain experimental conditions favor virus unmasking, or the exact relationship between M/L viruses and host cells. A few fundamental studies of this type have been done. For example, adenoviruses can be isolated from adenoid tissues which are cultivated in vitro but homogenates of similar original tissues usually contain no detectable virus. There is some reason to believe, however, that fully infectious virus is present in such tissues, but not in readily detectable concentrations. Work with adenoassociated satellite virus and distemper virus suggests that these two viruses can exist in a truly masked form and are completely lost if the viability of the infected cell is lost. Human diseases which have been studied extensively to determine whether viruses are in any way associated include: Kuru, subacute sclerosing panencephalitis, progressive multifocal leukoencephalopathy and Jakob-Creutzfeldt disease. Convincing evidence is accumulating from transmission studies in higher primates, in vitro cultivation of brain tissues and electron microscopy of affected tissues, that M/L viruses are etiologically associated with each of these diseases. The recent reports on the isolation of new papovaviruses in humans, particularly in the brain, have revived interest in the possible role of this class of viruses in chronic human disease.

Using some of the techniques, referred to above, a program to study explant cell cultures from human and simian brains is conducted with an attempt to determine the presence or absence of masked and latent viruses which might be suspected in viral etiology. It is hypothesized that if a group of brain tumors with common antigen(s) can be identified, these might be candidates to examine for a viral etiology. It is hoped to develop a serological test which will detect putative antibody responses to such antigen(s) in the tumor-bearing patients. If such antibodies exist and can be detected, then larger groups of normal subjects and patients would be tested.

Criteria of the responses of the experimental animal to viral infection are established by careful monitoring of the physiologic behavior of the CNS following inoculation with tissue extracts and whole cells derived from brains of patients with chronic encephalopathies. The early events of the experimental diseases in animals have been detected by electroencephalography because the EEG record becomes abnormal before any other signs of diseases appear. These techniques are being perfected for clinical application. However, at present they permit the selection of diseased animals for study during initial phases of the pathogenic process. Before drawing any definitive conclusions about the nature of the disease produced, especially about its relationship to the original human disease, it is well to remember that a CNS disease produced in animals may not be identical with the disease of the patient from whom the tissue was obtained. A dramatic example of species difference in host response is the effect of simian virus B. In man it induces a disease that is severe, and usually fatal, whereas for monkeys this virus is innocuous.

Subacute sclerosing panencephalitis (SSPE) is a degenerative neurologic disease of children and young adults. It is characterized by progressive mental and motor deterioration, myoclonic jerks, and coma. The patients become severely emaciated and die from intercurrent infections. The diagnosis established during the incipient stages often shows a personality disorder or mental retardation and the EEG shows slowing and dysrhythmia. However, high amplitude, low frequency synchronous waves do not develop until the patient exhibits myoclonic jerking. Spinal fluid proteins and cell counts remain normal or increase slightly during the entire course of the disease. Transmission of encephalomyelitis from humans to animals and further from animal to animal, producing symptoms typical of SSPE in the animal, has provided an important new lead in isolating and understanding the causative agent in SSPE. During the last few years, evidence of a relationship between SSPE and measles virus has been established.

Measles virus, once considered primarily a respiratory disease agent, has within the last few years been recognized as an agent capable of producing a variety of pathologic states within the CNS. Measles has long been known to produce an acute type of CNS inflammation with subsequent demyelination during so called "post infectious encephalomyelitis," a condition in which the pathogenic mechanisms are still unclear. In 1966, new evidence showed that an agent antigenically identical to measles virus was etiologically involved in the chronic childhood disease SSPE. Convincing serologic, morphologic and virologic information has since shown that each case of SSPE contains a variant of measles virus. Increasingly, serologic data has accumulated suggesting that measles is also etiologically implicated in many cases of multiple sclerosis (MS). To date it has been impossible to isolate measles from MS patients and the pathogenesis of the disease still remains unclear. Nevertheless, such information indicates that measles virus commonly infects the CNS of man and, at least in some cases, remains in latent form to produce remitting or chronic disease months or years after the primary infection.

In order to better understand the pathogenic mechanisms by which measles virus reaches and remains within the CNS, a variety of animal models have been studied. A subacute disease in ferrets, dogs, calves, and monkeys was produced by inoculating the animals with cells containing the LEC strain of SSPE virus. During 1971 studies were undertaken with a strain of SSPE virus isolated from a human brain biopsy which was later adapted to the hamster CNS. This strain was labeled HBS virus. This virus produced an acute, universally fatal, giant cell encephalitis when inoculated intracerebrally into newborn hamsters but failed to propagate in extraneural tissues. Recently, an experimental chronic measles infection of the hamster CNS has been established which bears many of the virologic, histologic, serologic and ultrastructural characteristics of human SSPE. This model is now being used to answer some of the questions posed here: (1) What is the state of the defective CNS virus? (2) May the virus exist in one of several defective conditions? (3) How are measles antigens expressed during the defective infection and do they intermittently appear as in simpler in vitro systems? (4) What is the relationship of known measles antigens to structural viral components such as the "fuzzy" and smooth nucleocapsids? (5) What components of the host immune system appear and what are the relative functions of the humoral and cellular immune systems during chronic infection? (6) Can the latent infection stimulate the immune system to immunopathic destruction of neural tissue?

Five SSPE strains have been isolated from the brains of patients with SSPE and have been partially characterized. All these strains produced syncytia type cytopathology and grew to much lower titers than wild or vaccine strains of measles virus. Also, these strains were more neurovirulent for newborn hamsters and less reactive with antibody than non-plaque-purified reference strains of wild or vaccine measles virus. The latter observation suggests that they are not classic measles virus but are variants of measles virus of a similar nature.

Determination of properties of SSPE viruses is essential for elucidating why an individual develops SSPE and may be important in monitoring the safety of live-measles-virus vaccine. Biochemical studies have not yet revealed differences between SSPE and wild strain measles virus as regards their nucleic acids, the intracellular structures associated with virus-specific nucleic acids, or the response of these nucleic acids to treatment of infected cells with cycloheximide. None of the antiviral compounds tested discriminated between SSPE and wild strain virus. Virazole, the only one of these compounds which was effective in inhibiting measles virus, is currently being evaluated by some investigators as a therapeutic agent in SSPE. The above results, and all other known characteristics of SSPE viruses and epidemiologic evidence appear to be consistent with the hypothesis that SSPE viruses are variant strains of measles virus. More detailed biochemical studies of the nucleic acids of SSPE and other strains of measles virus could be greatly facilitated by using cycloheximide to stimulate synthesis and accumulation of virus-specific mRNA in infected cells.

Recent reports that homologous viral proteins inhibit in vitro transcription of RNA of Sendai or vesicular stomatitis virus (VSV) suggest that cycloheximide may stimulate synthesis of measles mRNA by suppressing synthesis of viral protein which normally inhibits virus-specific transcription. The observation that cycloheximide does not inhibit synthesis of measles nucleocapsid RNA is in sharp contrast with previous reports that protein synthesis is required for synthesis of virion RNA of paramyxoviruses and of VSV. This observation may indicate only that the RNA-replication enzyme of measles virus is more stable than those of paramyxoviruses or VSV. However, it is also possible that a fundamental difference exists between the mechanism for replicating RNA of measles virus and that for replicating RNA of other negative-strand RNA.

The exact relationship of SSPE virus to measles virus may be defined only after detailed biochemical and genetic studies. Reports have appeared which describe some chemical and physical properties of measles virus RNA and of RNA and nucleocapsides from measles virus infected cells. The comparisons of virus specified RNA's in measles infected cells with those in SSPE virus infected cells have revealed thus far only quantitative differences among six distinct species of RNA. Studies are underway comparing measles and SSPE viruses in terms of virion RNA and peptides, as well as virus specified peptides of infected cells. Mutants of measles and SSPE virus have been isolated which are temperature sensitive and which have other markers that will serve in both biochemical and genetic investigations.

Although the etiology of multiple sclerosis is obscure, epidemiologic evidence has suggested a common exposure factor, most likely a virus, in those afflicted with this most prevalent human demyelinating disease. Studies of viral causes of demyelination have, in the past, taken two general directions (1) in vivo and in vitro studies of animal viruses; (2) attempts to isolate virus from human diseases such as multiple sclerosis, Schilder's disease and progressive multifocal leukoencephalopathy (PML), a disease in which papova virus-like particles has been observed by electron microscopy. The study of animal viruses causing demyelination has been limited by the paucity of experimental models. Work has centered on two known viruses, JHM, a neurotropic strain of mouse hepatitis virus, and progressive multifocal leukoencephalopathy.

JHM virus-induced demyelination in mice appears to be the most practical model to study. In 1949, Cheever and co-workers isolated a murine virus causing a disseminated encephalomyelitis with extensive destruction of myelin. The JHM virus also caused liver disease and subsequently has been grouped with the mouse hepatitis viruses (MHV) designated MHV-4. The demyelinating lesions produced by acute infections with the neurotropic strain of mouse hepatitis virus were found to be dependent on dose of virus, age of mice, and the route of inoculation. Fluorescent antibody studies showed that the antigen was most prominent in cells of the white matter and immunosuppression studies gave no indication that there was an immunopathologic mechanism in the demyelination. Electron microscopic studies were initiated on animals after the acute phase at a time when virus could no longer be recovered but when there was still an active inflammatory response. Initial studies in these animals as long as one year after the acute infection have shown the presence of coronavirus virions in oligodendrocytes and what appears to be continuing demyelination.

Progressive multifocal leukoencephalopathy (PML) is a subacute to chronic demyelinating disease of man, much more rare than multiple sclerosis but of world-wide occurance. The disease infects adults from the third decade and later and occurs usually as a late complication of general body malignancies, especially of chronic leukemias and lymphomas, or of benign diseases of the reticuloendothelial system. Early in the disease, lesions in the form of aggregates of pin point sized grey foci appear in the immediate subcortical white matter. Later, they enlarge, coalesce and spread to involve most of the white matter of one or even both hemispheres. A unique cytopathology is the main criterion by which the disease is distinguished from multiple sclerosis and other demyelinating diseases.

From the standpoint of human disease, attempts to transmit multiple sclerosis and Schilder's disease to experimental animals has, thus far, been unsuccessful. In vitro studies, although poorly documented, have also been futile. Progressive multifocal leukoencephalopathy (PML), the only human demyelinating disease clearly associated with a virus has been of great interest. This subacute process, usually occurring in patients with incompetence of their immune response, has been characterized by multifocal neurologic deficits. Patients have no fever or cerebrospinal fluid abnormalities and the course is progressive, ending in death. Multiple foci of demyelination surrounded by oligodendroglia have been found in the brain. Many of these cells have inclusion bodies containing papovavirus-like particles. Attempts to isolate virus remained unsuccessful until recently when a virus which appeared unrelated to previously recognized papovavirus was isolated as an SV40-like agent from two cases of PML. The nucleic acids of the two SV40-like viruses isolated from patients with PML have been compared with the DNA of laboratory strains of SV40. The DNA of these isolated are both covalently closed circular duplexes with a sedimentation rate the same as SV40 DNA. The denatured DNA hybridized with denatured SV40 DNA at the same rate has homologous DNA. When digested with Hemophilus influenza restriction endonuclease, the DNA of both the SV40-PML viruses yielded 11 fragments but in each case there were two fragments which differed from the classic strains of SV40, and these two fragments from the SV40-PML viruses differed from one another.

Biological characterization of the SV40-like viruses isolated from PML were also carried out. Both showed remarkable stability to chloroform, pH 3 and heating at 50°C for 120 minutes. Both viruses were shown to grow efficiently in BSC-1 and primary African green monkey kidney cells although SV40-PML 2 virus showed more efficient replication, and the viruses differed in their plaquing characteristics. Both agents caused transformation of rodent cells, and in Wi-38 human cells there was apparent semi-permissive infection with the production of infectious virus but also cell transformation. Serum and kinetic neutralization tests showed complete cross reaction of SV40 virus. Tumor, "T", antigens appeared identical to SV40. No tumors were induced in mice inoculated with the viruses, but tumors were produced in hamsters resembling the tumors induced by classic strains of SV40 virus.

These studies have given further information on the mechanisms by which virus may produce demyelinating disease both in experimental animals and man and represent some detailed characterization of the papovaviruses associated with progressive multifocal leukoencephalopathy, the first chronic human demyelinating disease demonstrated to be of viral etiology.

Progressive multifocal leukoencephalopathy is caused by papovaviruses, but their exact identity and significance in producing demyelination still require clarification. A strain has been isolated for which antibodies were detected in 60% of randomly collected human sera of all ages. This strain is distinct from polyoma and SV40 viruses. Tumors have been successfully induced in the brain of hamsters after intracerebral inoculation of this human papova virus. The research plan now includes; morphologic identification of the DNA in this and other papovaviruses by means of the Kleinschmidt spreading technic; identification of viral antigens by electron microscopy; isolation and characterization of papovaviruses derived from other cases of PML; morphologic studies on the formation of brain tumors induced in hamsters by human papovavirus; and comparative studies of polyoma, SV40, and papovaviruses from other laboratories.

Transmissible mink encephalopathy (TME) is akin to scrapie, kuru, and Creutzfeldt-Jakob disease, i.e., the noninflammatory, subacute spongiform viral encephalopathies. These diseases develop over a period of months and years. The infectious agents are still unidentified, but their properties are distinct from conventional viruses. A study is being undertaken including sequential, morphologic study in hamsters and monkeys inoculated with TME brain suspensions in order to determine the localization and precise ultrastructural alterations that develop in neurons, particularly in synaptic regions, months before the onset of clinical symptoms. The proposed TME study is important in that the disease mimics human Creutzfeldt-Jakob disease. Thus far, all ultrastructural studies have dealt with the advanced stages of the disease in man and animal, but the elusive agent could conceivably be visualized earlier. The changes in the plasma membranes that appear to initiate the spongiform alterations in neurons warrant in depth study, for speculations concerning abnormal membranes are supported by biochemical data on the infectious agent.

Herpes simplex viruses, Type 1 and 2, are the cause of life-threatening encephalitis in the United States today. The real frequency and clinical spectrum of the disease is not fully known. Studies are limited by the current necessity of isolating herpes simplex virus (HSV) from the brain in order to make a definitive diagnosis. In preliminary studies, passive hemagglutinating antibodies (PHA) in cerebrospinal fluid have been found early during the courses of several patients with HSV encephalitis and with Herpes virus hominin encephalitis. This PHA-CSF technique will be evaluated as an immediately available diagnostic test for HSV encephalitis. Cerebrospinal fluid PHA will be correlated with isolation of virus at brain biopsy and with measures of conventional and complement requiring neutralizing antibodies in sera and CSF during various neurological conditions. Diagnostic studies will be augmented by in vivo and in vitro studies of the pharmacology of potentially useful antiviral agents in experimental models and in man. An in vitro assay of antiviral activity in body fluids due to administered drug which is not affected by interferon or homologous serum antibody has been developed which includes concentrations of idoxuridine in serum, urine and cerebrospinal fluid of patients with suspected diagnoses of Herpes virus hominis encephalitis. Minimal inhibitory concentrations of agent vs strains of HSV will be correlated with amounts of drug in blood, spinal fluid, brain and urine after therapy. Possible antiviral synergy of several antiviral drugs in combination will be assayed. These data will guide rational therapy in man.

A number of viruses have been implicated in the etiology of multiple sclerosis. It is surprising that vaccinia infection, almost universally distributed, has not been sought as a possible agent. However, no vaccinia studies of multiple sclerosis have been done with the exception of electron microscope searches for elementary particles which have been unsuccessful. It is clear that, if vaccinia antibodies are found in a significant number of spinal fluids of multiple sclerosis victims and in no other chronic neurologic disease, this might be of some diagnostic use for neurologists faced with early states of the disorder, such as optic neuritis, which may or may not progress to the full-grown picture of multiple sclerosis. More important, if vaccinia can be related to etiology, a significant reduction in incidence of routine vaccination may lead to a reduction in this disease. A study is being conducted on the frequency of the presence of neutralizing antibodies against vaccinia virus in the spinal fluids of victims of multiple sclerosis and of other patients suffering from chronic brain syndromes. Preliminary evidence suggests that only multiple sclerosis patients have such antibodies in the spinal fluid. In order to relate the possible etiologic significance of this finding to the disease itself, it is proposed to do fluorescent antigen studies on brains of multiple sclerosis victims using specific antibodies against four soluble antigens of vaccinia (NP, S, HS, HL).

Visna is the only model of slow, virus-induced demyelinating disease in animals. However, work on this infection has been limited because the disease could only be produced in Icelandic sheep. Previous attempts to adapt visna virus to other breeds of sheep or other animals have not been successful. Because of the importance of this model to study demyelination relative to multiple sclerosis, further attempts have been made to adapt the visna virus. Preliminary studies have been carried out using the rapid serial passage of visna virus in fetal sheep. Visna was originally recognized in Iceland as a naturally occuring fatal central nervous system disease of sheep and was described by Sigurdsson as one of the prototypes of slow infection. The causal agent is now classified as an oncorna virus, based on its physical, biochemical, and biological properties. Following intracerebral inoculation of susceptible Icelandic sheep, a persistent infection develops followed by a late appearance of neutralizing antibodies.

This study postulates that the disease is immunopathological in nature and will attempt to elucidate (A) the cellular sites of virus replication; (B) the role of the immune response (antibody and cell-mediated) in disease production and (C) the mechanisms of virus persistence. Approaches will include (a) a sequential study of infection in Icelandic sheep; (b) the effect of immunosuppression, using antilymphoid serum of cyclophosphamide; (c) the effect of active immunization before and after inoculation; (d) the correlation of various measures of antibody and cell-mediated immunity with disease progression; (e) a search for evidence of an auto-immune response agains myelin antigens; and (f) the influence of age of inoculation upon the course of infection.

Major pathogenesis experiments so far conducted indicate that nearly all sheep injected intracerebrally with strain 1514 of visna virus got infected over a varying period of time up to 12 months. Virus was frequently present in CNS, spleen, and lymph nodes and less commonly in other sites. All sheep developed pathologic lesions, particularly severe periventricular inflammation. Further evidence of infection was obtained from blood and CSF, complement fixation test and neutralizing antibody responses. These results are encouraging in that the production of visna-mediated CNS disease is more reproducible in frequency and severity than has ever been seen in the past. Much of the future work involving electronmicroscopy, immune response and immunosuppression is now being carried out on this reproducible model.

A possible relationship between experimental allergic encephalitis (EAE) and visna virus is being investigated. The pathology of EAE, as described for animals such as the guinea pig and rat bears considerable resemblance to visna in the prominent nature of the inflammatory reaction, its cellular composition, and the subsequent destruction of CNS parenchyma, particularly of the white matter. Furthermore, the slowly developing lesions in visna suggested the possibility that sensitization to CNS antigens might be initiated during the prolonged incubation prior to clinical signs. These arguments suggested that the hypothesis of an auto-immune component in visna should be investigated as one aspect of this study. The strategy selected was first to produce EAE in the sheep, and if this proved readily feasible, to set up assays for antibody cell-mediated responses to basic protein and other CNS antigens. These tests could then be applied to visna-infected animals to search for evidence of an autoimmune phenomenon.

The possibility that visna infection initiates an auto-immune process is being studied by production of experimental allergic encephalitis (EAE). Work to date has documented that acute EAE can be readily induced in sheep, which develop a fulminating and fatal form of disease, a finding not previously described. This observation has stimulated an intensive effort to develop _in vitro_ correlates of the immune response, to quantitate both antibody and cell-mediated immunity.

Results to date indicate that affected sheep have very severe inflammation throughout the CNS, mainly due to infiltrates of lymphocytes and monocytes. Complement fixation antibody tests have been developed, using both basic protein and an alcohol extract of brain. Most sheep show a rising serum titer to both of these antigens following sensitization. Also a considerable proportion have pre-sensitization antibody to basic protein, a result requiring further evaluation. Using basic protein, peripheral blood leukocytes undergo a lymphocyte transformation response when taken from sheep with acute EAE. Application of these tests to visna-infected sheep has so far failed to reveal evidence that there is an antibody response to basic protein during the course of infection. However, the presence of pre-infection basic protein antibody in considerable numbers of sheep and the limited time of follow-up (3-5 months) precludes definite conclusions. Less extensive tests for lymphocyte transformation have so far shown normal indices in visna-infected sheep.

Visna is a slow progressive demyelinating disease of the central nervous system of sheep caused by a virus which shares many biological properties with the RNA tumor virus. The proposed research will examine (a) the mechanisms of persistence of virus; (b) the pathogenesis of the demyelinating lesions in the brain and spinal cord, (c) the effects of altered host immune competence on the outcome of infection. The study is divided into three parts: short-term studies in infected young adult lambs, long-term studies in the same group of animals, and infection of thymectomized fetal lambs. On the basis of pilot experiments, studies will be directed to (a) identification of infected cells using virologic techniques both at the cellular and molecular levels, histochemical tests including immunofluorescence, immunoperoxidase, and autoradiography and electron microscopy; (b) the lymphoproliferative aspect of the disease using virologic and a variety of immunological techniques; (c) the humoral and cellular immune responses to the infection and the pathological effects, or lack of, in infected immunologically compromised lambs.

Initially, visna virus was cultivated in primary sheep choroid plexus cells and primary sheep testis cells following methods of Sigurdsson. A plaque assay has been set up and an antibody neutralization assay for the virus as well and have demonstrated North American lambs to be susceptible to intracerebral infection with the virus with production of clinical symptomology and pathological changes similar to the field disease reported in Iceland. Fetal infection was also accomplished with evidence of persistence of virus in brain and lung for periods up to twelve weeks. Four-week-old lambs yielded virus from brain explants but not from cell-free tissue homogenates. Perivascular changes in the white matter were noted together with a mild, focal meningitis and an interstitial pneumonia. Lymphocytes react to virus in two ways. They were specifically sensitized in vivo to visna virus antigen and responded to antigen with blastogenesis in vitro. Lymph node cells were shown to be infected by visna virus in vitro with subsequent stimulation of DNA synthesis but without significant replication of virus.

Investigations are being conducted to understand the intricacies surrounding the replication of visna virus nucleic acid species and how these synthetic processes relate to the ability of this slow virus to persist in susceptible host tissue until overt clinical symptoms appear. The formation of visna virus DNA intermediates designated as "DNA provirus" will be studied in cultures of sheep choroid plexus cells, and the presence of these virus-specific DNAs will be detected by testing the DNA fraction of infected cells for its capacity to stimulate reassociation kinetics of denatured DNA generated in vitro by reverse transcriptase or by testing for its integration within "network" DNA formed by Britten-Kohn repetitive sequences under conditions of self-annealing. The aim in these experiments is to determine whether visna "provirus DNA" occupies a specific site on a host chromosome and associates with a given size class of cellular DNA. The effect of various inhibitors of protein and nucleic acid synthesis on the formation and integration of these visna DNA species will also be examined.

Other major projects included in the research plan are molecular investigations of viral-specific nucleic acids and proteins in diseased tissue of sheep infected with visna virus and a search for resident gene products of covert slow virus genomes in human autopsy specimens of brain and other organs from victims of multiple sclerosis. The latter tests will focus on demonstrating viral nucleic acids having sequences homologous to visna virus by reassociation kinetics of visna transcriptase product, by DNA-RNA hybridization studies with labeled visna RNA and by use of complementary "minus" strand DNA generated by the visna transcriptase for implicating the presence of viral RNA transcripts in cell fractions.

Ultrastructural investigation of viral infections involving the central nervous system of experimental animals and man is being conducted. Emphasis is being placed on disorders in which a viral infection may be associated with demyelination or with a teratogenic effect. Human biopsy material from chronic degenerative disorders of the CNS of suspected viral etiology will also be screened electron microscopically for detection of a possible agent. Specifically, cerebellar malformation induced by the Kilham rat virus, and the effect of the blue tongue virus on the cerebral cortical development will be examined. The study of the demyelinating disease induced in mice with the JHM strain of mouse hepatitis virus will focus on two specific questions, namely, the mechanism of demyelination and the role of the immune response in demyelination, and the source of cells involved in remyelination. Human studies will consist of a continued attempt to determine the occurrence and frequency of viral isolates in progressive multifocal leukoencephalopathy, and electron microscopic monitoring of cultures derived from patients with known and suspected viral disease of the nervous system.

The objective of the multiple sclerosis (MS) virology program is to isolate infectious agents from tissue obtained from patients with multiple sclerosis and to characterize them and determine their relationship to the disease. The rationale for a virologic approach to the study of multiple sclerosis is based on the fact that some clinical features are common to both multiple sclerosis and the known slow virus diseases. Serologic and epidemiologic information tend to incriminate a viral agent as the cause of multiple sclerosis. The recent isolation of a virus (6/94) from the brains of two patients with multiple sclerosis lends further support to the viral etiology concept. The multidisciplinary approach to the MS problem (clinical, virological, immunological), combined with the newer laboratory techniques (cell fusion, use of halogenated pyrimidines, immunosuppression) to activate viruses in tissue culture and in experimental animals, offers a better chance for the discovery of a virus than has previously existed.

Multiple sclerosis fits the three criteria established by Sigurdsson for slow virus diseases (a) after infection, a period of many months or years without signs of disease; (b) after clinical signs appear, a protracted course, usually ending in serious impairment or death; (c) infection limited to a single host species and lesions limited to a single organ system. Thus, MS is probably acquired but not manifested during childhood, runs a protracted course, seems to be species specific for man and attacks initially only the CNS.

Some of the known slow virus diseases of man (kuru, Jakob-Creutzfeldt and SSPE) and animals (scrapie, visna, transmissible mink encephalopathy and Aleutian disease of mink) have been shown to be transmissible to susceptible hosts. Although several studies have been conducted to induce or propagate these virus with MS-tissue extracts, the efforts so far have remained unsuccessful. On the other hand inoculation of animals with cells or tissue extracts obtained from cases of SSPE, progressive multifocal leukoencephalopathy, kuru, Jakob-Creutzfeldt, mink encephalopathy, scrapie and visna resulted in the production of disease. Only with two viruses, SSPE and PML, was it possible to demonstrate and characterize a virus or its components inside CNS cells. With tissue obtained from Jakob-Creutzfeldt cases, it has been possible to relate the presence of a virus to morphological transformation in both the original brain cultures and in cells derived from cultures exposed to the original brain cells.

Recently, after brain cells from two cases of MS were fused with indicator cells, an agent referred to as the 6/94 virus was isolated. This agent has now been propagated in embryonated hens eggs. Hemagglution-inhibition tests with egg-grown 6/94 virus permitted it to be differentiated from both the HA-2 and Sendai agents; two prototypes of parainfluenze viruses to which 6/94 is related. Using physicochemical techniques such as purification, determination of molecular weight, sedimentation co-efficient, analysis of RNA species and electrophoretically-distinguishable protein migration, 6/94 virus has been further studied and compared with the related Sendai virus. The 6/94 virus inoculated intracerebrally into newborn Syrian hamsters caused severe clinical and pathological syndrome. On the other hand, HA-2 virus produced severe hydrocephalus but failed to cause the marked wasting characteristic of 6/94.

6/94 virus has been analyzed for its antigenic, RNA and polypeptide compositions and for selective biological properties in animals and cell cultures. The results establish that 6/94 virus contains a 50S RNA genome and is related to Sendai virus in its antigenic and total polypeptide compositions. Despite these similarities, the 6/94 and Sendai viruses differ in certain phenotypic properties. 6/94 virus is markedly less cytocidal for chick fibroblasts, especially at 37°C and after B-propiolactone inactivation possesses a greater capacity for cell fusion and a lower toxicity than does comparably-treated Sendai virus. In addition, 6/94 virus shows greater hemolytic activity. Polypeptide analysis of egg grown 6/94 virus and Sendai virus on SDS-polyacrylamide gels revealed that each virion possessed at least 8 polypeptides. The major polypeptide associated with each of the viruses (M.W. 60,000 daltons) was found to be associated with the nucleoprotein. Both viruses possessed glycoproteins with molecular weights of 68,000, 52,000, and 48,000 daltons.

It has been pointed out earlier that in two chronic CNS diseases, visna and PML, a link has been suggested between slow virus infection and viral oncogenesis. The rationale in searching for a similar correlation in MS is supported by the observation that the 6/94 agent is different antigenically, biochemically and biologically from more than one of the parainfluenza I prototype viruses and argues against it being a laboratory contaminant. Also, the presence of nucleocapsids in brain cells of one MS case before fusion, the appearance of virions upon fusion of these cells with both indicator cells (CV-1 and W138) and lack of virions and nucleocapsids in non-MS brain "fused cultures" argue against the indicator cells being the source of virus. The isolation of a similar (6/94) virus from the second case of MS lends further strength to the possible association of this agent with multiple sclerosis.

Brain tissues obtained at the time of biopsy or autopsy are being studied by light and electron microscopy in order to document and define any morphologic alterations and to search for viruses in tissue sections from patients with multiple sclerosis. These studies also include electron microscopy of tissue culture cells derived from nervous systems of patients with MS, EM of supernatant fluid from cell cultures to search for viral particles, morphological characterization of virions, morphological analysis of tissue to define unitype cultures and morphological characterization of 6/94 virus to augment the viral isolation studies and to define virus nucleocapsid in cells prior to fusion and before other tests for the presence of virus could be considered positive.

In general these programs are designed to achieve the following objectives (a) to obtain a viable MS tissue; (b) to investigate a variety of environmental parameters in search for the optimum conditions for the growth of human central nervous tissue in cell culture and their characterization; (c) to continue attempts to isolate, rescue and identify virus(es) from tissue cultured cells using standard and newer virologic techniques; (d) to further characterize the 6/94 virus, (e) to study immunological reactions in MS patients.

During the past four decades, an obscure disease of sheep called "scrapie" has emerged as an important area of biochemical research. Transmission studies and neuropathological examination of brain tissue suggest that scrapie is a prototype for the spongiform encephalopathies of man, i.e. kuru and Creutzfeldt-Jakob disease. Prior to the transmission of kuru and Creutzfeldt Jakob disease to chimpanzees by intracerebral inoculation of infected brain, both of these diseases were classified as "degenerative" abiotrophies of the nervous system.

To date the chemical nature of the scrapie agent remains obscure. Its unusual physico-chemical properties and its slow replication in the absence of any detection by host defense mechanisms suggest that it is a novel infectious entity. Unlike viruses, the scrapie agent cannot be visualized by electron microscopy and its presence does not provide a detectable immunological response.

This research program is directed toward understanding the chemical structure of the scrapie agent. Because the present assay for scrapie requires determination of an endpoint by titration in mice over 9 months, it is planned to devote effort to the search for a more rapid and less expensive biological assay. A reliable method for the partial purification of the scrapie agent is planned and this preparation would be used to explore chemical and immunological assay systems. The purification and subsequent elucidation of the chemical structure of the scrapie agent promises to bring new concepts and techniques to several areas of molecular biology and medicine.

NEUROMUSCULAR DISEASES

The muscular dystrophies (MD) and myasthenia gravis (MG) constitute the most prevalent neuromuscular diseases affecting approximately 200,000 (MD) and 30,000 (MG) individuals in the United States alone. The predominent symptom is muscular weakness. However, while MG is attributable to a failure of neurochemical transmission at the myoneural junction, the various dystrophies are primarily genetic in origin and are variously attributed to deficiencies in muscle proteins, muscle membranes, nerves, capillary circulation, and endocrine regulation.

Myasthenia gravis results from a defect in transmission across the neuromuscular junction probably due to either the inadequate release of acetylcholine from the nerve terminals or from a decreased sensitivity of the motor endplate to the transmitter. The size of the miniature endplate potentials arising from the spontaneous continual release of acetylcholine from the nerve endings is greatly reduced. There is also evidence that MG may be an autoimmune disease resulting in antibody interference with neuromuscular transmission. Drug therapy in MG is based either upon increasing the available acetylcholine at the junction or upon the suppression of the autoimmune mechanism. Anticholinesterase agents are used to decrease the rate of destruction of the neurohumor at the endplate and ACTH and corticosteroids are presumably effective through suppression of the immune process. The theophylline derivative, oxtriphylline, has proved effective in patients refractory to anticholinesterases.

Muscular dystrophy includes a group of diseases characterized by progressive wasting of skeletal muscle, primarily in children 2-6 years of age. The condition is usually inherited, may be sex-linked (Duchenne type), transmitted as a dominent trait (facio-scapulo-humeral) or may demonstrate autosomal recessive inheritance (limb-girdle type). A varient MD of late onset may occur in both men and women after the age of thirty. The "white, fast" muscle fibers are primarily affected while the "red, slow" fibers are relatively resistant. In the case of the Duchenne type, patients and 70 percent of female carriers usually show high levels of serum creatine phosphokinase, demonstrate excessive synthesis of collagen by isolated polyribosomes of muscle, and reveal a larger than normal percentage of red blood cells with abnormal membranes under the scanning electron microscrope. There is no accepted treatment for MD other than physical therapy although unconfirmed reports suggest that the administration of high oxygen, glucagon, or coenzyme Q are beneficial in the dystrophic mouse.

Involvement of the immune system in MG had previously been suggested by the occurrence of thymic hyperplasia, the presence of antibodies directed against muscle structural proteins, the presence of lymphocytes toxic to muscle tissue, and the beneficial effects of repeated lymphocyte drainage. In the past year, a number of different laboratories have produced paralysis in laboratory animals by the injection of purified muscle receptor protein. The animal model, similar to MG in pharmacological response to neostigmine and electromyographically, has been reproduced in rabbits, rats, goats, and monkeys. Antireceptor antibody of rabbit sera block the carbamylcholine response of the monocellular electroplax preparation, precipitate receptor protein solubilized from cloned rat muscle cells, and may block the electrophysiological response of rat muscle to nerve stimulation. The globulin fractions of the serum of some MG patients block the binding of alpha bungarotoxin to receptor protein of isolated muscle membrane preparations. Alpha bungarotoxin specifically binds to the acetylcholine receptor in muscle.

Using biopsies of myasthenic muscle, it has been shown that diseased tissue has only 11 to 30 percent of the binding capacity of normal muscle for alpha bungarotoxin. This finding raised the question of whether receptor reduction alone could explain the pathophysiological defects of MG. Blockage of acetylcholine receptors by the intravenous administration of cobra alpha toxin to rats resulted in physiological and pharmacological changes typical of MG, including decremental responses to repetitive stimuli, curare sensitivity, neostigmine reversal, and post-tetanic phenomena. In summary, evidence is accumulating that MG may be an autoimmune disease resulting from antibody blockade of postsynaptic acetylcholine receptors.

Clinical research involving the treatment of MG patients refractory to the usual medication has followed two lines. Alternate day prednisone at high dose levels is apparently helpful to approximately one-half the patients; double-blind studies are continuing. Derivatives of theophylline have also proved effective in refractory patients. Additional, potentially more efficacious compounds are being evaluated. Animal studies suggest that these drugs act both pre- and postsynaptically. They increase the frequency and amplitude of miniature endplate potentials, increase the probability of acetylcholine release, increase quantal size, and increase the amplitude of the endplate potential.

Circumstantial evidence implicates the muscle surface membrane as a possible site of the basic genetic defect in MD. Red blood cells from patients afflicted with the three major types of MD, as well as carriers of the X-linked Duchenne type, exhibit dramatic surface deformations when observed with the scanning electron microscope. It had previously been shown that the membranes of red blood cells obtained from dystrophic patients differ from normal ones with respect to fatty acid and phospholipid composition. Subtle changes in the adenosine triphosphatase activity and potassium efflux have been noted, possibly also reflecting membrane alterations.

Adenyl cyclase, a sarcolemmal enzyme, shows decreased activity in preparations obtained from human dystrophic muscle. If this abnormality is also expressed in tissue culture, it will provide additional evidence of a genetic fault in the muscle membrane. Among other properties, adenyl cyclase is required for the conversion of inactive lipase to its active form. In the absence of active lipase, triglycerides would not be converted to fatty acids (required for energy metabolism) and would lead to the accumulation of fat. In Duchenne dystrophy, lipase activity is relatively low, but it is not yet certain that this results from the diminished activity of adenyl cyclase.

Another potentially important abnormality noted in Duchenne muscle is concerned with the sarcoplasmic reticulum, the vesicles intimately related to muscle contraction through their capacity to accumulate and release calcium within the muscle cell. Using fragmented sarcoplasmic reticulum preparations, studies of the kinetics of calcium transport, total capacity for calcium accumulation, and calcium concentrating ability, leave little doubt that this system is significantly affected in Duchenne dystrophy.

Developmental aspects of MD in animals continue to provide important clues. To determine whether the inherited MD of the chicken is neurogenic or myogenic in origin, limb buds from normal and dystrophic chick embryos were exchanged prior to muscle differentiation and innervation. Bicept muscles of hatched chicks, in which muscles of the donor were innervated by nerves of the host, were analysed for embryonic properties of muscle acetylcholinesterase and fiber diameter, two distinctive markings for the expression of the dystrophic gene. The results indicate that MD of the chicken is caused by an initial biochemical lesion in its muscle rather than in its innervating nerve. In genetically dystrophic mice, light and electron microscopic analyses indicate that the earliest morphological signs of this disorder are found in the swollen sarcoplasmic reticulum. No abnormalities were observed until day 19 (shortly before birth), suggesting that in this instance the process of early muscle development was unaffected by dystrophy. The B10 14.6 line of Syrian hamsters is being used to determine whether exercise modifies the course of MD. The incidence of MD in this animal is 100 percent and it is presumed model for the Duchenne type, exhibiting muscular weakness and ataxia after 60-120 days of its 150-280 day life span.

In clinical studies, it has been shown that the administration of diethylstilbestrol (DES) to boys with Duchenne Dystrophy retards loss of muscular strength and lowers the abnormally high serum levels of various enzymes such as lactic dehydrogenase (LD) and creatine phosphokinase (CPK). The latter effect of DES is apparently due to the decreased efflux of LD and CPK from the diseased muscle, suggesting a primary effect of the drug on the sarcolemma. Ribosomal protein synthesis in preparations derived from Duchenne dystrophic muscle is greater than normal. Excessive collagen synthesis accounts for most of the additional protein and the same phenomenom is observed with muscle from female carriers of the X-linked Duchenne dystrophy. The protein synthesis test coupled with serum CPK determinations can detect over 90 percent of carriers.

CONVULSIVE DISORDERS

Nature of Epilepsy

Although epilepsy has been known for centuries, it is still somewhat difficult to define the disorder in terms which will cover its manifold features. Yet it does seem obvious that epilepsy is not one clearly defined disease but rather is a syndrome. The syndrome is characterized by a persistent liability to recurrent, paroxysmal (sudden, episodic) attacks. In the majority of these attacks there is impairment of thought and awareness or responsiveness, or both, and there may or may not be convulsive movements or automatisms. In attacks in which the disorder remains localized, consciousness may not be impaired. The seizures may be associated with structural disorders of the brain, or with a toxic cause. More frequently, however, there is no discoverable organic disease of the brain with which seizures are associated. Some forms of epilepsy may be genetically determined.

Classification

The clinical manifestations of seizures are so varied that an entirely satisfactory classification is difficult to make. In general, there are two ways of classifying patients with convulsive seizures. The first method classifies patients into two groups, symptomatic and idiopathic, according to the presence of known organic factors which may be of importance in the occurrence of attacks.

The second method of dividing patients with seizures is that of separating them into several groups according to the manifestations which occur during the attack: (1) Grand mal or generalized seizures; (2) Focal seizures - Jacksonian and generalized seizures with a focal onset; (3) Petit mal or minor attacks; and (4) Psychomotor attacks. Jacksonian and generalized seizures with a focal onset usually occur in patients with a structural lesion in the nervous system and are less common than the other forms of seizures. Of the other three types, grand mal seizures are the most common if the number of affected individuals are considered. Petit mal seizures are the next most frequent and psychomotor attacks are the least common. It is not uncommon for a patient to have two or more types of attacks. If the number of attacks is considered, petit mal is the most common type because it is not unusual for many attacks to occur daily in patients who are subjected to this form.

Symptoms

In the classical form of the "grand mal" seizure, the attack consists of an aura which is followed by a shrill cry, loss of consciousness and generalized and clonic movements. During the convulsive phase of the attack there may be tongue-biting, urinary or fecal incontinence and, rarely, ejaculation. "Jacksonian and focal" seizures occur almost exclusively in patients with an organic lesion in the cortex. The seizure starts with convulsive twitchings of one portion of the body, usually the distal part of one extremity. If the seizure starts in the fingers, the spread is to the wrist, the forearm, arm, face, and then to the homolateral leg. If the movements spread to the opposite half of the body, consciousness is lost and the further manifestations are similar to those of a grand mal seizure. "Psychomotor" epilepsy is caused by discharging neurons which exert their influence upon mental processes as well as upon muscle movements. A patient exhibits adversive or torsion movements, dreadful fear, flinging of arms aimlessly, smacking of lips, and other incoherent physical and mental behaviors. "Petit mal" seizures are a manifestation of epilepsy in childhood and rarely, if ever, have their onset after the age of twenty. The syndrome of petit mal consists of a triad of symptoms: myoclonic jerks, akinetic seizures and transient absences or loss of contact with the environment. In some patients, the attack may last only a second or two. In others, the attacks are of longer duration, lasting from fifteen to ninety seconds.

Etiology

Epilepsy may be caused by several factors, such as brain damage, presence of a scar caused by a wound or injury, drugs, congenital malformation, nutritional deficiencies, metabolic abnormalities, fever (in infants), infectious diseases (encephalitis, meningitis), brain tumors and abscesses.

During the past year, many heretofore unknown facts concerning the nature of epilepsy have been reported by researchers supported by the National Institute of Neurological and Communicative Disorders and Stroke. One investigator is directing an epilepsy research program using various approaches within the disciplines of neurophysiology, neurochemistry, neuroanatomy, clinical neurology and electroencephalography to investigate (1) the effects of epileptiform discharge on the developing brain; (2) basic abnormalities in cell-cell interactions in epileptogenesis; and (3) certain areas in clinical epilepsy.

Recent accomplishments by this group of researchers include the evaluation and treatment of patients with intractable epilepsy. In selected cases, implantation of epidural electrodes and subsequent surgical resection of focal zones of epileptogenic discharge was performed. The clinical electroencephalographic investigations included detailed EEG analysis of epileptiform activities in several categories of patients not carefully evaluated before. This work led to clinical recognition of a characteristic group of orbitofrontal epilepsies and the discovery that mesial temporal epileptiform discharges occur in high incidence in a group of patients with alcholism subject to attacks of violent behavior. Development of techniques of power spectral analysis and coherence measurements of human EEG furnished an important methodology for the study of problems of normal brain development and analysis of abnormal brain function as might occur in epilepsy or in diffuse cerebral insults produced by trauma, hypoxia, hypoglycemia and other metabolic stresses.

Another study by a second group of researchers involves the interrelationship of neurotransmission to neurological disease. These researchers are presently developing tools for the precise ultrastructural localization of macromolecules important in neurotransmission in an effort to define their interactions in biochemical physiological and pharmacological terms, and to apply this knowledge to the understanding and management of epilepsy and other neurological disorders. Using sophisticated immunocytochemical techniques, it has been possible to visualize glutamic acid decarboxylase, the rate limiting enzyme in biosynthesis of γ-aminobutyric acid (GABA) in light and electron microscopic preparations of the CNS. This accomplishment represents the first successful ultrastructural localization of an enzyme that is rate limiting in the synthesis of a known neurotransmitter.

Using experimental animal models of epilepsy, another group of investigators contributed significantly to the understanding of factors involved in the normal and abnormal functioning of the mervous system. By studying normal and abnormal structure relations in experimental animals, and in humans where possible, comparisons were made between normal and epileptic cortex allowing new concepts of seizure production.

Since astrocytes show the most marked change of all cells in the alumina epileptic focus, this gliosis was studied for comparison with non-epileptic scars. In control scars, Cajal stains showed a small run of reactive astrocytes, but in animals with alumina foci, the gliotic reaction extended far beyond the cortical injection site. Ultrastructural alterations consisted of increased astrocytic cytoplasm containing filaments and increased numbers of filament attachment plaques to the inner aspect of the plasma membrane, glycogen and microtubles. Connections to other astrocytes by tight junctions, electronic, and dense desmosome junctions were profuse in the experimental groups. These were also seen in controls, but less often. Astrocytic membrane connections of the desmosome type of junction to dendrites were also observed. It was concluded that these contacts, astrocyte and astrocyte to neural elements, may represent the anatomical counterpart for the electrical syncytium proposed by some physiologic studies.

The same investigators also studied Na, K ATPase activity and diphenylhydantoin concentration in the human epileptic brain. This study was done in conjunction with chemical studies on tissue routinely removed at surgical operations. In human epileptic foci without significant neuron loss and with marked astrogliosis, a significant decrease in Na, K ATPase activity and diphenylhydantoin concentration were found. It was concluded that the astrocytic proliferation being the main anatomic change implicated this scarring process as being either responsible for or intimately involved in the underlying deficiencies.

One laboratory has conducted studies designed to provide information regarding the mechanisms of generation and control of epileptiform activity in the brain. Particular emphasis was given to the effects of projected focal cortical epileptiform activity upon the behavior of subcortical neuronal aggregates and the relay of information along afferent pathways. A second goal was to continue investigations of the cellular mechanisms involved in generation of acute epileptiform discharges produced by convulsant drug application to the cortex.

The results from these studies include recordings from relay cells in 35 cats obtained during focal strychnine or penicillin-induced epileptiform discharge in precruciate cortex. Thalamocortical relay (TCR) cells were identified by their orthodromic responses to stimulation of brachium conjuntivum and antidromic activation following cortical stimulation. During focal epileptiform discharges induced both by penicillin and strychnine the predominent event in TCR cells was the generation of an inhibitory postsynaptic potential (IPSP) lasting as long as 400 msec. In some neurons excitatory postsynaptic potentials (EPSP) were superimposed upon the IPSP, particularly during repetitive low frequency activation of the interictal cortical discharges. During the tonic phase of spontaneous or triggered ictal episodes, rhythmic EPSPs replaced the IPSPs and repetitive impulse generation resulted. In the clonic phase of the seizure large amplitude depolarizations with high frequency superimposed spike bursts occurred.

During interictal discharges about 35 percent of identified TCR cells generated bursts of spike potentials which were proven to originate in intracortical axons. Intracellular and quasi-intracellular recordings showed that antidromic bursts invaded TCR cells in the midst of the IPSPs described above. Interictal antidromic spike bursts in VL relay neurons were much less frequently encountered when strychnine rather than penicillin was used to create the cortical focus.

These findings are important because they indicate that the phenomenon of antidromic spiking is not restricted to a particular thalamic relay nucleus or to epileptogenesis produced by a particular agent. They also show that transmission of sensory information to the cortex may be interrupted by focal epileptiform discharge.

Studies of field potentials and unit activity in cuneate neurons during penicillin epileptogenesis in postcruciate cortex were also conducted. The results of these experiments indicate that interictal epileptiform discharges produce high frequency input in fiber pathways to cuneate neurons. The predominant effect is generation of IPSPs in cuneate relay cells. However, depolarization of dorsal column afferents in cuneate also takes place during each interictal epileptiform event suggesting that presynaptic inhibitory circuits are also engaged by the cortical efferent discharges. Inhibition on cuneate relay cells appeared to persist during ictal episodes, rather than be replaced by excitation, as in the case of TCR cells in VL.

These results demonstrate the widespread effects of focal epileptiform discharge on synaptically related neuronal aggregates. In a sense, the epileptogenic neuronal aggregate in sensory cortex, through actions at the cuneate and thalamic relays, functionally deafferents itself during each interictal event. This may provide a mechanism for controlling the frequency and spread of discharge, but at the same time the widespread inhibition interfered with normal neuronal activities at distant sites.

Another investigator has been conducting neuropharmacologic studies in experimental epilepsy. These studies have helped to delineate the antiepileptic action of procaine-HCl and lidocaine-HCl on electrically-induced after discharge thresholds and on penicillin-induced epileptiform activity. It was shown that this antiepileptic action of local anesthetics occurred in both intact and in brain stem transected animals and that the presence of the cerebellum is not necessary for the antiepileptic action of the drugs. It appeared that the local anesthetics exert an antiepileptic action which, although its mechanism is unknown, differs from that of diphenylhydantoin or phenobarbital.

These investigators also conducted a series of physiological studies which delineated the responses of the cerebellar cortex and the dentate nucleus to the various stages of penicillin-induced epileptiform activity. The primary significance of the studies is the observation that, while cerebellar Purkinje cells cease discharging during development of epileptiform activity which becomes synchronous and maximal in both cerebral hemispheres, the dentate nucleus does not cease firing during these prolonged bouts of epileptiform activity. These observations are in general agreement with the working hypothesis that cerebellar inhibitory discharge (via Purkinje cells) plays a role in the modulation of cerebral cortical excitability.

Preliminary evaluation of four compounds of potential therapeutic usefulness in petit mal epilepsy have been made. They included imipromine, clonazepam, tranxene, carbamazepine. Of these agents, imipramine and carbamazepine were found to be the least effective. Tranxene was only partially effective. On the other hand, clonazepam was a potent suppressant and was found to be more active than diazepam. From these results, it appeared that clonazepam may be of the greatest usefulness in the clinical treatment of petit mal epilepsy.

In an exhaustive pharmacologic analysis of the potential antiepileptic usefulness of carbamazepine (Tegretol), the drug was tested against premarin-induced 3/sec spike and wave activity, against penicillin-induced cortical epileptiform bursts and on after-discharge thresholds (all in cats), and against alumina cream-induced epileptiform activity in Rhesus monkeys. Carbamazepine was found to effectively suppress penicillin-induced epileptiform activity in cats and alumina cream-induced activity in monkeys. It raised the threshold for electrically induced cortical discharge in cats. Therefore, the investigators consider the compound to be of greatest use in those laboratory models which correlate well with major seizures while they consider it to be less effective in minor seizures.

In acute studies, blood levels of 3.0 to 10 mg/ml were necessary in order to suppress epileptiform activity, while in chronic foci in monkeys, blood levels of 2 mg/ml or lower were effective in suppressing both electrical and clinical seizures. The investigators felt that due to the poor solubility of carbamezapine, the blood brain barrier may be a significant hindrance to the transport of the drug into the brain in an acute situation. It was concluded, therefore, that carbamazepine may be better suited to long term management of the epileptic patient than to short term therapy. The mode of the antiepileptic action of carbamazepine was not elucidated.

Brain ribosomes and brain protein synthesis in epilepsy is being investigated in another laboratory supported by NINCDS. The overall objectives of these investigations are: (1) the analysis of the biochemical mechanisms by which epilepsy affects the brain protein synthesis and (2) the study of potential application of this knowledge to medical problems, particularly regarding the effects of seizures in the immature brain.

Brain protein synthesis during epileptic seizures was measured _in vivo_ using male rats. The animals were atropinized to prevent arrhythmias, paralyzed with succinylcholine to prevent convulsions, tracheotomized and ventilated with 100% O_2 on an animal respirator. Seizures were induced electrically at specific intervals.

Because of the use of multiple controls and because the experiments were the first in which anoxemia and convulsions were totally avoided, it is probably that these studies measured the effects of seizures more than any previous experiments. The results showed a profound inhibition of protein synthesis in all brain regions tested. The extent of inhibition increased with the duration of seizures. The specific activity of the free leucine pool was elevated by seizures. The investigators suggested that this elevation may have been due to increased blood flow.

Two other studies conducted by this laboratory involved the effects of seizures on brain development. One concerned the effect of neonatal seizures on brain development, the other concerned the role of malnutrition in seizure-induced brain damage in neonates. The first study compared three groups of animals grouped at birth in threesomes of the same litter, sex and body weight: (1) rats subjected to two daily electrical seizures; (2) rats subjected to the same total amount of electrical current at subconvulsive intensities and (3) untreated controls. All rats were killed at 30 days of age. In seizure-treated rats, brain development was impaired (reduced DNA and cell number) and a number of behavioral milestones were delayed while "current control" rats were similar to untreated controls in all respects.

The second study compared rats subjected to electrical seizures twice a day to littermates of the same sex and birth weight that were separated from the mother for variable periods in order to keep their body weights the same as that of seizure-treated rats. The degree of malnutrition was the same in both groups since both animals of each pair had similar birth weights and similar body weights on each day of life until sacrifice (30 days). It was found that the brain weights and brain DNA were smaller in the seizure-treated rats than in their malnourished controls. It was concluded from these results that the developmental effects of seizures are specific for seizures and not the result of anorexia or malnutrition.

Another group of researchers have been working on cerebral methylations in normal and convulsive states by examining in some detail the effects of the convulsant agent methionine sulfoximine (MSO) on cerebral methylation reactions. Experiments were designed to test whether the administration of MSO induces significant changes in the ratio of S-adenosylmethionine/S-adenosylhomocysteine in various parts of the brain which can be related to its convulsant properties. These researchers also investigated whether nervous tissue of MSO-treated animals contains larger amounts of methylated amine metabolites than does control tissue. Finally, they studied the methylated bases of ribosomal RNA in the nervous tissue of control and MSO-pretreated animals by administering radioactive methionine _in vivo_ to both groups of animals.

Results from the studies conducted by these investigators showed that: (1) Methionine antagonizes MSO with respect to its uptake by tissues, particularly the brain and the spinal cord; (2) the relatively uniform concentration of MSO reached throughout the nervous tissue suggests that no brain region takes MSO up and sequesters it preferentially; yet a trend toward its initial accumulation by the cerebellum, suggested previously as the primary target site of the drug, and the spinal cord could be domonstrated; (3) Methionine not only antagonizes the uptake of MSO, it also markedly reduces the amount of MSO fixed to cerebral structures; (4) MSO found in bound form in the spinal cord is less susceptible to release by Triton than is MSO bound to brain structures. The latter observation, along with the finding of relatively high levels of MSO in the spinal cord points to a possible involvement of the spinal cord in the pathophysiology of the MSO seizure.

During the past year, these investigators also examined the possibility that the convulsant action of MSO may involve serotonin (5-HT) metabolism. Laboratory observations showed that MSO seizures are abolished both in mice and in rats by the administration of 200 mg/Kg of 5-hydroxytryptophan and are exacerbated by the administration of p-chlorophenylalanine. In a study of methionine sulfoximine -5-hydroxytryptophan interaction, changes in the brain levels of the rate-limiting 5-HT precursor, L-tryptophan were determined. There were virtually no alterations of tryptophan levels after MSO in brain regions examined. However, when the effect of MSO on the p-chlorophenylalanine-induced tryptophan depletion was tested, a potentiation of the p-chlonophenylalanine effect was noted in the cerebral cortex but not in the rest of the brain. It was found that 5-HT levels after MSO are limited to selected brain regions and, in addition, are dependent, both in location and intensity on the dose and schedule of MSO employed. Also, it was possible to demonstrate under conditions of convulsant as well as subconvulsant dose administration of MSO, significant reductions in 5-HT in selected brain regions.

Experiments were also conducted in which the effect of MSO on 5-HT turnover was examined. This was done by measuring 5-HT levels in seven brain regions after administering depleting doses of p-chlorophenylalanine together with MSO and comparing to 5-HT levels after p-chlorophenylalanine alone. This experimental design, used by others to study drug effects on 5-HT turnover, revealed the following: MSO potentiated the depleting action of p-chlorophenylalanine in the hypothalmus and the midbrain and it antagonized it in the hippocampus. MSO was without effect in the cerebral cortex, the cerebellum, the brainstem and the striatum.

COMMUNICATIVE SCIENCES

All of the communicative activities of the individual are products of his nervous system. The normal functioning of the individual's nervous system, its associated sensory system and associated motor system are necessary to normal communication. Therefore, any injury, disease or developmental delay of the several systems would cause a disturbance of the individual's communicative capacities.

So-called abstract thinking involves meanings which are generalized, applying to a class of situations never to a particular situation. The tool of abstract thinking is the concept. A concept is not to be understood as any particular structure, however. A concept may be any psychological process whatsoever which performs the function of carrying generalized meaning. Students of the development of concepts have found them to be closely related genetically to the development of language. The primary function of language is to communicate meanings to others. These meanings may be concrete and particular or they may be abstract and general. The important point is that in the developing of a system of signs or symbols to convey meanings to others, man has at the same time furnished himself with an indispensable tool to use in manipulating meanings in his own thinking. It is improbable that any great development of abstract or general thinking could have taken place without the possession of the language tool. Language, even in concrete perceptual situations, strips off the unessential factors and seizes upon the important element. A study of the historical development of written language shows that it has gradually changed from a system of concrete pictures of objects to a very schematized code. Spoken language has probably undergone some analogous process.

Oral speech and picture language coalesced to form one language system, when drawings and graphic signs were employed to represent, not objects, but sounds in the oral speech system. The result is the capacity to express thoughts schematically. This in turn reacted upon thought, so that by inner speech it is possible to think schematically.

Language, defined as a system of expressive signs has, therefore, acquired a double function: that of conceptual analysis and synthesis and that of communication of meanings.

The current year has seen a number of advances in the several sciences and clinical investigations supporting our knowledge of communication and the disorders which impair it.

Hearing and Deafness

In an investigation on the mechanical properties of the ear a group of researchers looked at tympanic membrane preforations. They found that when monitored by controlling sound pressure in front of the tympanic membrane, there is a 12 dB/octave loss with inverse frequency; when monitored by controlling the voltage across the transducer, the loss in the middle frequencies is attenuated; when monitored by controlling the sound pressure at the entrance of the ear canal, the low frequency loss is frequency independent; the low-frequency loss (12 dB with inverse frequency) is explained by the fact that after tympanic perforations the net pressure across the membrane decreases with 12 dB octave toward lower frequencies.

This group developed a powerful bone conduction vibrator and completed several bone conduction experiments. They report that acceleration when monitored at the bone conduction input is not a good indicator of BC energy entering the head; the sound pressure generated in the external ear canal by the BC signal is a good indicator for frequencies below 2KHz; using BC/AC cancellation techniques, it became possible to separate the bone conduction component of the external, and probably, the middle ear from that of the inner ear in the way predicted by earlier experiments in cats. Further research in this same laboratory on the laser interferometer developed in this laboratory and used to study tympanic membrane displacements was modified, making it less sensitive to vibrations both structural and air-borne. Methods are being worked out for opening the basal cochlear turn, placing a micro-mirror on the basilar cochlear membrane, and sealing the opening, before conducting any measurements. These steps are necessary to achieve a perpendicular approach to the basilar membrane which is necessary for the interferometric technique. Previous data, except by Bekesy, utilized the Moessbauer technique which does not require perpendicular incidence. Although the absolute error might not have been serious (-1 dB for an angle of 26 degrees), it is possible that the magnitude of read-out varied with frequency depending on whether the curvature of the membrane when being displaced was predominantly longitudinal ($f>fo$) or transversal ($f<fo$). The interferometric method should help clarify this point, since it is only able to measure the magnitude of the displacement component perpendicular to the plane of the membrane. It is also superior to the Moessbauer method with regard to sensitivity and dynamic range.

In an investigation of the psychophysics of binaural hearing, a team of investigators require human subjects to detect or to lateralize dichotic signals that contain interaural time differences or interaural level differences or both. While they are still unable to develop a quantitative account of the way the human auditory system combines these two types of information, they have succeeded in obtaining data that no current theory can handle, and this fact is finally becoming appreciated by the developers and adherents of those theories. Their findings relate to an experiment on masking level differences (MLDs) at high frequencies. Past research has shown MLDs to be primarily a low-frequency phenomenon (below about 1000 Hz), where the auditory nervous system is sensitive to cycle-by-cycle time differences. The small MLDs (approximately 3 dB) seen at higher frequencies have been attributed to the interaural level differences available in the waveform typically used for these experiments. In their experiments they used waveforms that allow them to control the interaural level differences in a way not possible in the typical MLD experiment - namely, a particular value of level difference can be presented through the observation interval of a single trial and across all of the trials of a block. When this was done at high frequencies, they were able to obtain MLDs of 6-12 dB instead of the typical 3 dB. When only an interaural time difference was made available at high frequencies, detection performance was essentially identical to that in the corresponding non-MLD condition. Thus, the idea that high frequency MLDs are based on the available interaural level differences was confirmed, and further, they showed that if the interaural level difference was unchanging throughout the trial, the binaural system yields an MLD several times larger than that obtained with the typically used waveforms. While doing pilot work for the above experiment, they discovered that changes in the bandwidth of the narrow-band signals they were using drastically altered detection performance in the non-MLD conditions, but had no effect on the MLD conditions. The effect was large and, as far as they could tell from a quick look at the literature, undocumented and not anticipated by the energy detection models commonly used to account for performance in the non-MLD conditions.

In a group of investigations aimed at a better understanding of sensory processes by means of psychophysical and physiological experimentation and mathematical analysis and biostimulation, one group of investigators devoted the main effort on the neural processes in the cochlea, as seen through single-unit recordings in the auditory nerve. This occurred because of the somewhat unexpected discoveries that promise to bring them a substantial step closer to an understanding of cochlear processes. When Kiang's monograph, "Discharge Patterns of Single Fibers in the Cats Auditory Nerve," appeared in 1965, it seemed that coding of auditory information in the auditory periphery was almost completely understood. The only major problem remaining seemed to reside in the sharpness of the encountered tuning curves, as contrasted with the flatter distribution of the mechanical amplitude in the cochlea. The discrepancy has produced many hypotheses but, on the whole, did not seem too surprising in view of structural evidence for interaction among outer hair cells connected to the same spiral fibers. Spoendlin's discovery that about 95% of all afferent auditory-nerve fibers end on single inner hair cells suddenly changed the whole picture and made our concepts of the 1960's appear naive. The discovery means that practically all, and perhaps all, microelectrode recordings from the auditory nerve concern radial fibers ending on inner hair cells. Neither the inner hair cells nor the radial fibers seems to possess structures that would make an interaction among them possible. How then is the neural sharpening of the mechanical frequency analysis in the cochlea achieved? What do the outer hair cells and the spiral fibers do?

Experiments performed about a year and a half ago on this project strongly indicated that the inner and outer hair cells interact. Experiments performed this year strengthened this conclusion and confirmed the expectations that the interaction occurs via the spiral fibers. Whether these fibers send lateral branches synapsing directly on the inner hair cells or interact with the radial fibers at the entrance to habenula perforata via satellite cells is not clear. Current anatomical evidence is quite equivocal on this issue, and other unknown possibilities may exist. Preliminary experiments seem to exclude a direct participation of the olivo-cochlear efferent system, however. When the cholinergic synapses of this system are blocked, the tuning curves of single auditory nerve fibers appear unchanged. These researchers now have a standard method of recording from the auditory nerve fiber in mongolian gerbils (Meriones unguiculatus). The access to the nerve is gained through the bulla and a small cavity adjacent to the round window. Only a thin bone separates the proximal end of this cavity from the auditory nerve. The bone is pierced with a small pick and a microelectrode is advanced hydraulically through the resulting opening. Both gerbils with normal ears and with ears partially damaged by means of Kanamycin are used. They have been applying trapezoidal (at the cochlear level), sinusoidal, and click stimuli. The fundamental of the former is maintained at 40 Hz to clearly separate velocity and displacement components of the resulting neural responses. These responses are quite complex in animals with normal ears. Nevertheless, they were able to analyze them into two a.c. components approximately 180 degrees out of phase, plus a d.c. bias that appears to increase with the units' characteristic frequency (CF). The exact phase angle also seems to vary slightly but monotonically with CF. In the absence of corresponding outer hair cells the response patterns to trapezoidal stimuli become simple and agree with one of the predicted components. They indicate an excitatory response synchronized sometimes entirely with the displacement of the basilar membrane toward scala vestibuli, sometimes with the velocity of basilar membrane motion in the same direction, and sometimes with an intermediate phase of motion. It seems that inner hair cells in the less severely damaged cochleas tend to produce the displacement responses. On the basis of these and some additional experiments, it was concluded that the inner and outer hair cells do interact in phase opposition and, in addition, that the sensitivity of the inner hair cells is only slightly smaller than that of the outer hair cells.

In an investigation of perceptual masking a group of researchers define perceptual masking as the increased difficulty in understanding speech that occurs when background sounds are complex and include competing speech. Variables that affect the magnitude of perceptual masking in normal hearing and in hearing impaired were studied. The goal was to determine whether or not perceptual masking actually occurs. They could not proceed to evaluate perceptual masking in hearing impaired subjects until they were certain they had eliminated artifacts due to methods of defining signal intensity and that they had techniques which would allow them to quantify perceptual masking in normal hearing. This they have done with replication, refinements and extensions of previous efforts. They are now prepared to study masking in hearing impaired. The model of perceptual masking they have developed is that competing acoustic stimuli create an unsorted mixture of neural impulses flowing into the central nervous system through each auditory nerve. This incoming conglomerate can usually be separated into trains of information representing the original stimuli. The separation is organized around autocorrelational and cross correlational features arising from so-called physical cues. However, subsequent sections of the nervous system cannot cope simultaneously with all the incoming information. Filtering, therefore, occurs which gives preference to one train of information, allowing it to proceed to higher centers through a limited capacity channel. This train of information is then processed so as to be perceived. It becomes the focus of attention. Other trains of information concurrently penetrate to a lesser degree at best. Escape from masking may be viewed as a special case of the operation of the filter mechanism. Masking occurs when the filter mechanism is not receiving enough identifiable neural data about the obscured sound to allow those data to be assembled into a discrete information train that can be transmitted through the limited capacity channel. One would predict that a sequence of increases in masking can be created by introducing progressive computations into the filtering task. Such a sequence can be produced experimentally. It concludes several steps when speech (spondees) is used as the maskee. This year's effort progressed to the phase where speech rather than noise becomes the masker for target speech and when multiple talkers constitute the background.

The key study was a quantification of perceptual masking following development of a trustworthy technique for normalizing data to compensate for physical differences between noise masker levels and speech masker levels. This normalization was achieved by amplitude modulating (AM) white noise by one, three, five and ten talkers. The resultant AM signal was then band pass filtered via a filter having a spectral shape similar to that of five talkers. Consequently, the masking noise had the same temporal characteristics as the modulating speech signal. However, the maskers differed somewhat in spectral level detail. Therefore, the long term spectrum was measured for each masker and a correction factor computed for that masker. The correction factor was the average deviation from the contour of unmodulated speech spectrum noise, with this average computed in terms of the 20 "equally contributing bands" associated with the articulation index. The correction was applied to the speech reception thresholds for spondees of 20 young adults with normal hearing. These thresholds were established against backgrounds of one, three, five and ten talker speech signals; one, three, five and ten talker modulated filtered noises; and unmodulated filtered noise. Data for each masker were normalized to non-modulated filtered noise at 75 dB SPL. The mean spondee threshold for the unmodulated filtered noise condition was 66.6 dB. Filtered noise modulated by one, three, five or ten talkers produced masked speech reception thresholds (after normalization of 57.8; 62.6; 62.3 and 64.1 dB) respectively. Here the increase in masking as speakers are added demonstrates the filling in of acoustic windows. The masked thresholds obtained with speech signals were 60.9; 70.0; 70.8 and 70.1 dB for one, three, five and ten talkers, respectively. Comparing speech thresholds to speech-modulated noise-masked thresholds, it can be seen that speech signals produce 3.1 dB more masking for one talker, 7.4 bB more masking for three and five talkers and 6 dB more masking for ten talkers. The slight reduction in masking effectiveness with 10 talkers may be the result of the composition of the 10 talker signal; five talkers forward combined with the same five talkers backward.

Cochlea-Inner Ear

In an attempt to improve our understanding of sensorineural deafness, a group of researchers are exploring the inner ear pathology that underlies it. During the past year they have continued their work on the cochlear pathology associated with aging as well as the effects of noise and ototoxic drugs in man and animals. Atrophy of the stria vascularis appears to play a key note in all of these conditions. It is generally accompanied by degeneration of the spiral ligament. In man the process appears to start at the basal end of the cochlea, but in aging dogs, as well as in the young Dalmatian, atrophy starts at the apex instead. In either case it appears to be progressive, and to precede the loss of hair cells. Strial atrophy is also a prominent feature of hereditary cochlear degeneration in the waltzing guinea pig, despite a Swedish report to the contrary. Studies of the blood supply of the inner ear have confirmed in the squirrel monkey the same features of the strial blood flow previously seen in the guinea pig. There is a high hematocrit in the strial vessels, which are larger than the other cochlear capillaries. Afferent and efferent vessels are closely inverted by the processes of the marginal and intermediate cells, and there is no actual pericapillary space as seen in the spiral ligament and elsewhere in the cochlea. The strial vessels, unlike renal glomerulus and those of the modiolus recently described by Kimura, are not fenestrated.

In general, strial changes tend to be more extensive than actual loss of hair cells, especially in man. In trained monkeys after exposure to intense sound and treatment with ototoxic drugs, there has been a close correlation between the final audiogram taken immediately before sacrifice and the location and extent of hair cell loss. Strial changes have tended to be more widespread. The macaque sacrified immediately after a 40 hour exposure of 120 dB octave band noise centered at 2 KHz where they included large vacuoles in the marginal cells, and capillary endothelial swelling which occluded some of the strial vessels. Strial pathology including vacuoles and partial loss of osmiophilia was seen in patas monkeys that were severely deafened by treatment with dihydrostreptomycin (DHSM) 10.0 mg/kG daily for three months. Hearing loss for frequencies about 1-2 KHz has also been followed as it has developed in monkeys treated with DHSM at only 40 mg/Kg daily for three months, a dose equivalent to 2 gm/day for a 50 Kg tuberculous patient. The patas monkey is the only species in which they were able to produce a hearing loss with DHSM comparable with that reported in man. The group reported three cases of ototoxic deafness in man which show the same combination of hair cell loss, strial atrophy and incipient degeneration of cochlear nerve fibers as they found in monkeys and guinea pigs. The studies of the cochlear changes in sensorineural deafness indicate the vital importance of early diagnosis of this condition if there is to be any hope of arresting it by dietary or medical intervention (e.g., by the use of fluorides in capsular otosclerosis). The fact that the stria appears to be the primary target organ in so many types of deafness suggests the possibility of imporoving its chances of survival by preventing or controlling hyperlipoproteinemia, hyperglycemia and other unfavorable metabolic conditions.

In an investigation of response of inner ear to injury, a research team is studying the initial effects of various types of trauma on inner ear epithelium to elucidate the mechanisms by which cells lining the cochlear duct are damaged and how these injuries lead to cell death. They found the appropriate noise exposure which would produce minimal hair cell loss immediately after exposure, but total degeneration of the organ of Corti during the month following exposure to be an octave band of noise with a center frequency of 4 KHz and a sound pressure level of 108 dB. When the duration of the exposure is one hour and the ear is perfused within one hour after termination, no cell loss is found in the organ of Corti in the middle of the first turn. However, if the ear is perfused one month after exposure, a small segment of the entire organ of Corti is found to have degenerated in the first turn. In ears perfused within one hour after termination of the exposure, outer hair cells in the first turn are somewhat swollen below the level of the reticular lamina. Whorls of membranes of smooth endoplasmic reticulum (simular in structure to Hansen's bodies) are scattered in these cells. The rest of the organ of Corti have a normal electron microscopic appearance at this time. Specimens taken 3, 6, 12 and 24 hours post-exposure show sequential degenerative changes in the outer hair cells. Their plasma membranes appear thinner than normal by phase contrast microscopy. This was found to correspond to a decrease in the number of rows of cisternae in the peripheral membrane system. The nuclei of these cells became enlarged and pale-staining with chromatin clumped along the nuclear membrane. The cells become progressively more swollen and eventually ruptured. However, by electron microscopic standards, the apical surfaces of the ruptured cells were still intact at this time. Now the investigators are able to begin the studies of permeability in the reticular lamina, making use of electron microscopic tracers to test the hypothesis that some damage in the inner ear following noise exposure is the result of ionic changes within the fluid spaces of the organ of Corti which may be due to changes in the permeability of the junctions between cells at the level of the reticular lamina. Using the exposure described above, they should be able to identify some of the earliest morphological changes which occur in the ear following exposure and to determine whether or not permeability changes precede these cytological alterations. These investigators, looking at ischemic changes in the inner ear, found that the cochlea of several guinea pigs were made ischemic by severing the animals' aortae and quickly placing the animals in a nitrogen atmosphere at 37°C. Specimens were perfused at .5, 1, 1.5 and 2 hours after sacrifice. Non-mylinated nerve fibers within the organ of Corti were the first to show signs of injury. By phase contrast microscopy the radial tunnel fibers were swollen, vacuolated and discontinuous in ears that were ischemic for one hour. By electron microscopy it was found that the afferent nerve fibers in the inner spiral bundle were quite edematous and contained mitochondria with deranged cristae.

In a study of stimulus-related DC potentials of the cochlea, a team of investigators are attempting to quantify and identify the origin of the various summating potential (SP) components and a delination of the cochlear generator potential (GP). They developed a novel method of creating controlled, narrow cochlear lesions in order to assess the contribution of a very limited segment of the organ of Corti to the total recorded cochlear potential. This method consists of passing very high frequency (5 MHZ) current through the organ of Corti between two intercochlear electrodes. Depending on the total charge driven through the organ, the degree and spatial extent of the damage can be controlled. This method allows them to quantify what the output of a narrow segment (0.5-2.0 mm) of the organ of Corti is by taking the difference between pre- and post-current electrical responses. In experiments utilizing very low frequency sounds to mechanically bias the cochlear transducer they found that at lower stimulus bias levels, the SP components could be reversibly enhanced or depressed according to the magnitude and phase of the bias. At higher levels, the SP is generally depressed. This effect appears to be a manifestation of the interference phenomenon. The changes in the SP are very similar to those seen with electrical biasing. It is concluded that the mechanical biasing signal results in a biasing current flow through the organ of Corti and that this current produces the characteristic alterations in the SP. The results are consistent with their hypothesis that the source of SP production resides in nonlinearities associated with hair cell function in the guinea pig.

In an investigation of the structure and function of the inner ear, a team studied the innervation of the mammalian cochlea and the avain cochlea with functional implications of relevance to human hearing. They studied the entire organs of Corti of newborn guinea pigs stained by the Gogi method. Ten ears from newborn kittens have also been stained. There is some variation in the number of hair cells innervated by a single external spiral cochlear nerve fiber in any turn. However, in general, their original observation has been substantiated: the cochlear nerve fibers in the basal coil supply fewer hair cells than do those in the upper coil. It is difficult to differentiate between the sensory and the "efferent" nerve fibers in the apical-most coils. In order to better define the size and structure of the nerve endings in the newborn animals, electron microscopy is being performed on the infants. A better knowledge of the efferent pattern at this age would also be of assistance in identifying both types of nerve fibers. The Karnovsky method for staining of acetylcholinesterase and the Maillet zinc iodide stain, both of which seem to be specific for the efferent nerve fibers, have been used to study the efferent nerve pattern. In addition, this team studied by TEM and SEM, the ultrastructure of the vestibular hair bundles and their relation to the cupula on the otolithic membrane in guinea pigs, using osmium and ethylene glycol fixed specimens. Evidence for the attachment of the cupula to the planum semilunatum and to the ampullar roof was found. The apical ends of the crista hair bundles were attached to the cupula. In the otolithic membrane three different types of organization were observed (1) a loose irregular network of fibrils, filling the entire sub-otolithic space; (2) a semi-organized fibrillar sheath along the kinocilium; (3) a highly organized lattice-work on which the otoconia rest and which is linked to "2". Stereo-kinociliary (SKC) bands were found in all three organs at the apical ends of the hair bundles. The unit SKC-bond consisted of two parts (1) extracellular interciliary filaments of macromolecular size; (2)adjacent intracellular structure in the kinocilia. The SKC-bonding varied between cristae and maculae. In the cristae, usually only one unit was present between one stereocilium and one kinocilium. In the maculae, generally the units between the kinocilium and several stereocilia were paired. These new findings not previously found in mammals provide a new dimension in the mechanism of hair cell stimulation.

In a study of the biochemistry of prominent structures of the cochlea, a team of researchers studied the transmitter metabolism of the cochlear nucleus. A method was perfected to enable them to recognize and dissect out regions and cells from frozen dried sections of rat and cat cochlear nucleus. The procedures were as follows. After freeze-dried sections of the cochlear nucleus are prepared, every other section is fixed and stained with Thionin. The stained sections are not suitable for chemical analysis, but they permit recognition of histological features not visible in the unstained freeze-dried sections, such as the layered structure of the dorsal cochlear nucleus. The use of the drawing tube makes it possible to cut out pieces of the unstained freeze-dried section which lie within specific histological regions whose boundries can be determined in the stained section. This is accomplished by placing a stained and adjacent unstained section to be analyzed on the microscope stage and the cochlear nucleus outlines, as well as the distinguishable regional boundries, are drawn onto a piece of paper via a drawing tube. The unstained section is then placed on the stage and the drawing of the stained section is projected onto it through the drawing tube. The outlines of the cochlear nucleus can be seen in the unstained section and aligned with the drawn outlines. The drawn regional boundaries then closely approximate the locations of the boundaries in the unstained section.

This research group studied the histological distribution of acetylcholinesterase (ACH) and choline acetylase (ChAc) and of four putative transmitter amino acids (glutamate, aspartate, glycine and Y-aminobutyrate) in sections of the cochlear nucleus of rat and cat.

ChAc catalyzes the synthesis of ACh from acetyl-CoA and choline AChE catalyzes and breakdown of ACh to acetate and choline. They used radiochemical assays for these enzymes as worked out by McCaman and others. To measure ChAc, tissue was incubated with C^{14}-labeled acetyl-CoA and with neostigmine as an inhibitor of ChE and the C^{14}-labeled. ACh formed was isolated and measured by scintillation counting. To measure AChE, tissue was incubated with C^{14}-labeled ACh and with iso-OMPA as an inhibitor of nonspecific ChE, and the C^{14}-labeled acetate formed was isolated and measured by scintillation counting. The four amino acids were measured with enzymatic flurometric methods using oil well techniques and the procedure of enzymatic-cycling previously described.

Hearing Disorders - Otitis Media

Otitis media is one of the most common infections afflicting humans, yet it is one of the most poorly evaluated. A team of researchers is attempting to rectify this by a study of experimentally induced serous otitis media in animals. Since they wish to correlate information on the biochemical, histologic and audiometric changes, they selected the guinea pig as a model. In studying various agents they found that Staphylococcus aureus, strain 502A, produced infection in the bulla. The presence of the infection in the animals' ears can be monitored by direct examination of the tympanic membrane. A thickened, minimally hyperemic, mid-position tympanic membrane with gross infection of the middle ear and bulla was found in 100% of the animals. In order to determine more accurately the morphological characteristics of infection in the chinchilla ear, ears have been infected for various lengths of time, the animals sacrificed, and the temporal bones submitted to routine temporal bone histopathology.

In the preliminary biochemical findings in perilymph in experimentally induced middle ear infection by Staphylococcus aureus, it was found that there were significant increases of lactate dehydragenase activity. Histochemical studies of the round window membrane in progress reveal that there is an increase in hydrolytic enzyme activity, particularly alkaline and acid phosphatase in the fibrous middle layer of the chinchilla round window membrane after infection. In the human middle ear effusion, the research team tried to further differentiate biochemically thin serous fluid and think mucous fluid, analyzing certain biochemical parameters of these fluids and comparing with those of serum. They noted that large-molecular-weight proteins (ceruloplasmin, transferrin and complement C3) were not in higher concentration in middle ear effusions than in serum and that this might indicate that the passage of these proteins into middle ear effusions is limited even under the inflammatory conditions of the middle ear mucosa and vessels.

Functional assessment of the hearing in animals and humans exhibiting otitis media was considered necessary to observe threshold shifts occurring as a result of altered cochlear function. Since the primary locus of any cochlear involvement due to otitis media is presumed to be in the basal turn, close to the round window membrane, it was assumed that hearing for high frequencies would be most affected. Because of this it was felt necessary to test frequencies above the conventional limit of 8000Hz. Preliminary data on humans with unilateral otitis media, tested so that the normal ear could be a control, revealed a profound loss for the affected ear, which after treatment undergoes remission. There appears, however, to be some residual loss in the very high frequencies (14000-16000 Hz).

Speech

A team of researchers are utilizing cinefluorographic techniques to study speech articulation so as to obtain basic data on articulatory movements during normal speech production which will lead to a more complete and simplified model of articulatory functioning. Their objective is to (1) obtain descriptive data on the articulatory characteristics of various types of speech elements; (2) define in more detail overlapping characteristics of speech elements; (3) define the dynamic properties of articulatory movements; (4) use the data in the formulation of general principles which govern speech articulation processes. A study of velar movements and timing was designed to obtain more information on velar movements during normal speech production, particularly the path of velar movement, rates of movement and timing of movements in relation to tongue, lip and jaw movements. The results indicate that the path of velar movement is slightly curvilinear but can be described with one degree of freedom in the mid-sagittal plane. The close coordination of velar movements with the other structures particularly the tongue, indicate that other structural movements and positions must be taken into account in considering the cause of variation in velar movements. In a study of articulatory timing in consonant clusters, high-speed cinefluorographic films of three adult speakers were analyzed to determine the timing of articulatory submovements in /spr/, /spl/, and /skw/ sequences containing variously positioned word and morpheme boundaries. The data revealed that some articulatory events are separated by as little as 10 msec and that certain articulatory intervals are essentially unaffected by variations in the position of linguistic boundaries. These timing data are not generally consistent with models that assume feedback-loop controls of movements based on completion of preceding movements or contacts and suggest that the timing of sequential movements may be pre-programmed at the input to the system. In a study of articulatory dynamics of stutterers and normal speakers, the timing of articulatory movements was examined during non-stuttered productions of syllables by six stutterers and seven normal speakers. Results indicate that even perceptually fluent utterances of stutterers differ from those of normal speakers in terms of latencies between articulatory events, i.e., stutterers showed significantly larger latencies. In addition, the relationship between the timing of the lip and jaw movements also differed for the two groups.

Another research group attempting to contribute to a theory that describes the unique human capabilities for generating speech-sound patterns and for auditory processing of speech has used several approaches (1) acoustic analysis of speech; (2) modelling of the speech production process; (3) study of the activity of speech structures; (4) experiments in perception of speech and speech-like sounds. With regard to the studies of laryngeal activity, a study of excised larynxes has led to a detailed description of the surface wave that propagates upward along the medial surface of the vocal cord and across the superior surface. The speed of propagation of this wave has been determined and the slopes of the trajectories of particles at various points on the vocal cord surface have been established. The wave pattern results in a phase difference between the closure of the inferior and superior portions of the vocal cord and estimates have been made of the relations between the mechanical vibrations and the forces on the vocal cord surfaces due to aerodynamic factors. Indirect examination of certain aspects of normal laryngeal vibration has been carried out by analyzing the output of a small accelerometer attached to the external neck surface below the larynx. With proper interpretation, this signal indicates subglottal resonances, points in time at which the glottis opens and closes, and the acoustic coupling from the subglottal cavities to the trachea during the open phase of vocal cord vibration.

In the study of physiological modelling of speech, a computer simulated model of the tongue has been developed. This model represents the tongue in terms of a series of points located on the tongue surface and within the body of the tongue. These flesh points are connected to each other and to points external to the tongue by elements that simulate muscles and connective tissue. The muscles are organized according to known anatomical data. Initial studies of the behavior of the model have examined the static positions of the tongue model when various individual muscles are contracted, and have investigated in a preliminary way the patterns of muscle contraction needed to achieve certain vocal-like configurations. The model behavior is consistent with a theory that associates each phonetic feature (such as high, back, advanced tongue root, and constricted pharynx) with a simple pattern of muscle contraction, usually involving primarily a single muscle. This modelling effort represents an approach to the study of the motor control of speech and will be continued to include dynamic patterns of tongue movement. In experiments on temporally-segmented speech it was found that when a continuous speech message is broken up by inserting 200 msec silent intervals, its intelligibility depends on the duration of the resulting speech intervals. An experiment showed that it is not the duration of the speech intervals that determines intelligibility, but rather the amount of speech they contain (i.e., the number of phonemes, syllables, etc.). This result has implications for the properties of the short-term memory involved in the processing of speech.

Investigators in the area of voice quality and laryngeal frequency have developed a new method for obtaining air flow waveform at the glottis by inverse-filtering oral volume velocity. The purpose was to use this method to solve some questions that obscure our understanding of the role of the larynx during speech production and further develop the method to provide an accurate and convenient tool for investigating voice pathology, for linguistic investigations and for speech training procedures. This necessitated a re-engineering of the mask-transducer system used to measure oral volume velocity to improve high and low frequency performance and explore the nonlinear relationships between glottal area and glottal air flow during voicing, and the possibility for obtaining an inverse to that transformation in order to derive the glottal area function. During a visit to the Royal Technical Institute in Stockholm, the researchers developed an electrical "black box" model of the voice source for use in speech synthesis, and to explore by inverse-filtering some of the problems encountered in trying to formulate such a voice source model. This objective was chosen in order to combine the insight into the functioning of the glottal source provided by their inverse-filtering work with the sophisticated speech synthesis procedure being developed at the Royal Institute of Technology. Events proved that in order to be useful for inverse-filtering, a pneumotachograph mask measuring oral volume velocity must have a flat frequency response from zero to at least 1000 Hz.

To overcome deficiencies of the previous design, a multichannel transducer system which measures low, medium and high frequencies by different techniques has been developed for the mask. The new system combines good high frequency fidelity with good low frequency response and zero level stability. However, because of the complexity and expense of the multichannel system, a very simple and easily produced single-channel mask has also been constructed which used a specifically constructed differential pressure transducer having characteristics not commercially available. This simplified system is being tested. Instrumentation is completed for measuring the effect of subglottal pressure variations on the glottal waveform. A vibrator has been mounted on a special chair so as to contact the abdomen and lower sternum in order to provide externally induced oscillations in subglottal pressure. Speech synthesis experiments with a three parameter model of the voice source have shown that the transitions in the spectral composition of the voice source (other than changes in fundamental frequency) resulting from adduction or abduction of the vocal folds are significant in the perception of speech. In fact, their experiments indicate that [h] type sounds, though normally referred to as "glottal fricatives," are signalled more by changes in the spectrum of the quasi-periodic component of the glottal wave than by the presence of turbulence-induced random excitation of the vocal tract. They also found that when such turbulence does occur in the [h] sound, it may not be produced at the glottis.

Researchers on speech breathing mechanics are developing information on the mechanical behavior of the respiratory pump in normal and organically disordered speakers. Their goal is a comprehensive account of respiratory function in normal speech, an account of disordered speech where respiratory dysfunction is involved, and improved diagnosis and management of respiratory-based speech pathologies. In earlier work in volume-pressure analysis of the function of the rib cage wall, diaphragm and abdominal wall for utterance conditions involving lung volumes, loudness and body position, they found a general background adjustment of the chest wall during utterance. Current work examined the rib cage wall and abdominal wall in speech samples involving different levels of linguistic stress. The findings revealed that in the upright body position, both the rib cage wall and abdominal wall increase their muscular pressure inputs during heightened levels of stressing. The muscular pressure input from the two parts of the chest wall has been found to vary in relation to the magnitude of the stress input. For minimal stressing at conversational speech levels, the rib cage wall and abdominal wall show essentially equal muscular pressure increments for stressed elements. For marked stressing in conversational speech, however, the increment in muscular pressure provided by the abdominal wall exceeds that provided simultaneously by the rib cage wall. By contrast, in the supine body position only the rib cage participates in providing heightened muscular pressure for minimal stress contracts. As found earlier, only the rib cage wall is active in the supine body position for normal loudness speech production. The only time the abdominal wall participates in stressing in the supine body position is when stressing occurs during loud speech where the abdominal wall is active simultaneously with the rib cage. In general, when stressing is accomplished, the required increment in muscular pressure is provided by the part(s) which is active at the moment rather than the recruiting of activity from part(s) that are inactive. This strategy appears to take advantage of the fact that the muscles which are active are already somewhat shortened and can perform the muscular pressure change faster than were the subjects to call in other inactive muscles to aid in raising the alveolar pressure.

Language and Speech Disorders

A research team has been studying acoustic measures for detecting laryngeal pathology by developing a reliable method to facilitate both the early detection of laryngeal disease and the quantitative evaluation of laryngeal dysfunction and to relate acoustic parameters to the physiology of voice production, to pathological changes in the larynx and to the perception of voice quality. Specifically, the system they developed for digital inverse filtering of the glottal wave was used on the data to test quantitatively the increase in sensitivity in given acoustic parameters measured from filtered waveforms over those measured from unfiltered waveforms. A new parameter, analogous to signal-to-noice ratio, that was measured from the filtered waveform was also tested. The computer programs previously proposed have an efficient user-oriented digital analysis system that performs inverse glottal wave filtering on tape recordings of normal or pathologic voices. Briefly, these programs separate the components of the speech wave attributable to the laryngeal voice source from those attributable to other factors, particularly the resonance characteristics of the oral and pharyngeal cavities. For acoustic detection of laryngeal pathology, then, these programs give access to the laryngeal componant of the voice in a more or less pure form. The analysis can optionally produce two related waveforms (1) an estimate of the glottal source waveform; (2) a filter residue signal, which is approximately the first derivative of (1). A study using this analysis system was conducted on 10 normal voices and 10 pathological voices. They found that the "signal-to-voice" (S/N) ratio, defined as the ratio of the residue signals peak amplitude to its root-mean-square amplitude in the last half of a glottal period, is a sensitive indicator of pathology. An informal study was conducted in which an untrained subject was asked to categorize the waveforms into two piles. The result was 100% separation of normal from pathologic voices. They are pursuing quantitative means for duplicating such separation automatically. The purpose of the proposed study is to establish some objective acoustic indicators of laryngeal pathology. If such indicators were determined, it would be especially useful to the clinician, for the acoustic signal is easily accessible and easily recordable with relatively inexpensive equipment.

In a study designed to assess communication processes in aphasia, a group of researchers are attempting to determine the ability to understand written discourse, understand spoken discourse, and test a new visual communication method for severely aphasic patients. Findings revealed that in reading at the sentence level, there was no difference in overall ability to detect errors in written discourse between patients with anterior and posterior pathology. Anterior patients performed somewhat better on an oral version of the test and posterior patients on a written version. Examination of errors revealed a regular order of difficulty in sentences featuring contradiction to real world knowledge. The order of difficulty of sentences featuring deviations with no semantic force differed appreciably depending on the locus of the lesion. In oral reading of single words, it was found that part of speech and manipulability of the referent of a word made a significant contribution to ease oral production. This effect was found in tests of naming as well as reading. Patients with pure alexia proved able to read words which are short, irrespective of the part of speech; patients having alexia with agraphia proved more successful with picturable nouns, even when these were longer. The findings underscore the contributions of both syntactic and semantic features to oral reading. In a study of grammatical judgement, evidence indicated that the apparent dissociation between disrupted output and relatively preserved comprehension in Broca's aphasia is somewhat illusory. From metalinguistic judgements obtained from agrammatic aphasics, it would appear that, when syntactic features are poorly controlled on the level of spontaneous speech, they are unlikely to be preserved at other levels of language. Semantic redundancy in the surrounding sentence and rate of presentation made the most potent contributions to comprehensibility. Patients with posterior lesions proved especially prone to acoustic confusion. Patients with adequate comprehension at the start of testing displayed a uniform tendency to improve on conditions administered later, while those with more impaired comprehension showed no such improvement. In the study on comprehension of definite and indefinite reference, patients were presented sentences in which the inclusion of an article either hindered or aided in the specification of an intended referent. Anomic aphasics seemed sensitive to this distinction, while Broca's aphasics gave no indication of processing the article, whether or not it was appropriate to the extralinguistic situation. Thus, the inability of Broca's aphasics to mark definiteness to their spontaneous speech was parallel to their insensitivity to this feature in a task of comprehension.

Taste and Olfaction

A group of investigators are studying behavioral and neural effects of toxicosis and pain by investigating predatory control by aversive conditioning. Utilizing a method of taste-illness conditioning developed for laboratory animals, they tested a method of averting predators to domestic animals. The method was remarkably successful with coyotes. Coyotes given one or two meals of minced lamb followed by illness induced by lithium chloride refused to attack lambs on subsequent trials. They continued to attack rabbits, indicating the aversion was specific to lamb. Other coyotes treated with rabbit-illness conditioning refused rabbits but continued to attack lamb. Some species differences were noted. Ferrets treated with mouse-illness procedures continued to kill mice although they would no longer eat them. In contrast, the coyote would not kill prey that it was not prepared to eat. In a study of the role of olfaction in avoidance conditioning, previous research had demonstrated that rats could not effectively use odor to avoid poisoned food. Rats needed to taste the food, smelling was not enough to insure a strong aversion in a single trial followed by illness. While this is contrary to the popular notion that food flavor is mostly smell, it makes good sense neurologically. Taste afferents project to the n. solitarius which also receives fibers from the homeostatis monitors. Olfactory afferents do not. Olfactory fibers project to the limbic system and have no such direct connection with the n. solitarius. This led the investigators, to believe that odor cues are functionally suited to guide the rat toward external rewards and away from external punishments. They found that animals quickly associated odor with shock, but that they found it extremely difficult to use taste cues to avoid shock. Since olfactory fibers project into the limbic system, odors may have a primary function in sex arousal and this is being investigated.

A team of investigators are attempting to uncover the neural mechanisms utilized by the gustatory system to detect, discriminate and respond to taste stimuli encountered by animal organisms. To this purpose they studied the chemosensitivity in spiders, the intracellular responses of taste buds in frog and salamander and attempted to trace the pathway of the chorda tympani nerves of the cat through the medulla and pons. In the first study, the aim was to investigate the response of individual receptors on chemosensitive hairs and the possible interaction between them. The major advantage of this species of spider was the ability to apply chemical stimulation with a micropipette to an area restricted to only one hair. This proved an ideal control for stimulus spread when studying interactions. Feeding experiments were performed in 60 spiders to determine what stimuli were effective. Strong preferences were shown for fly or mealworm juice. In some cases sucrose and glycine produced positive response. Preference was measured by the amount of time the spider held on to a sponge rubber lure impregnated with the stimulus.

Neural activity was recorded from approximately 50% of all brains tested on 40 spiders. The most effective stimulus was NACl (from 0.1 to 0.5M). Two types of responses were seen. Type I had a peak impulse frequency of 5-20/sec. reached within the first five seconds of stimulation. Within one to two minutes the frequency declined to zero. Type II showed little activity initially and reached a peak frequency of 10-20/sec. after one or two minutes of stimulation. Repeated stimulation, even when interspersed with rinsing and rest periods, tended to depress the magnitude of the original response. Peripheral interactions were studied in 30-40 pairs of hairs from four spiders. In all cases no change in the impulse frequency in the first hair occurred following application of the stimulus to second hair. These results are in keeping with the idea that each receptor has a single dendrite in the hair and an axon that does not branch on its way to the CNS.

In the second study the major finding in the frog intracellular work is that antidromic stimulation of the afferent nerve leads to either hyperpolarization or depolarization depending on whether the cell is on the surface or lower layer of the taste disc. This is related to observations by others that taste stimuli may produce either a depolarization or hyperpolarization of the taste bud cell depending on the concentration of the stimulus,

In the third study on the cat, the aim was to trace the connections between the taste pathway and the gastric secretory neurons of the dorsal motor vagus nucleus. It has been established earlier that 2-deoxy-d-glucose produced gastric secretion through the reflex activation of a circuit originating in the lateral area of the hypothalmus (LHA) and they had postulated that the gustatory-gastric reflex might use the same circuit. They were not able to produce gastric secretion by electrical stimulation of LHA or of the chorda tympani nerve itself. However, in at least two cats they produced a marked response to stimulation of what may be a portion of the solitary nucleus. The problem here is knowing exactly the course of the gustatory fibers through the medulla. The reported ability of cut axons to take up cobalt dye may help to deal with this problem.

Tactile Perception

One research team is engaged in the study of tactile perception of speech. It is a program of research into vibrotactile information processing with a spcific goal, namely, the development of a vibrotactile vocoder for the perception of speech. Such a "tactile vocoder" could be valuable as a sensory substitute for the prelingual deaf. The research will attempt to explore certain areas where a possible theoretical roadblock to progress in vocoder research has been identified. Previous attempts to develop a tactile vocoder have not taken into account the limitations in temporal acuity and temporal short-term memory of the skin, as compared to the ear. The prime objective in this study is to explore the degree to which these limitations can be bypassed by the use of a "scanning" tactile vocoder that uses spatial acuity and spatial memory to augment temporal capabilities. In order to make better use of the information processing capacity of the skin, they are exploring the simultaneous use of stimulus frequency and location to convey frequency information and they are using stimulus waveforms other than sinusoidal. The new device has been constructed and is being tested. It includes a (1)treated room fitted for tactile psychophysics; (2) a specially constructed capacitance-type displacement monitor with wide linear dynamic range to monitor non-sinusoidal vibratory stimuli, (3) the electro-dynamic response characteristics of a Goodman's type 390A vibrator has been matched by a specially constructed "inverse-filter" so as to provide a flat system response to over 500 Hz. This response is required for faithful reproduction of non-sinusoidal waveforms, especially the pulse-like waveforms of interest to this study. Experiments in frequency matching require equalization of the subjective magnitudes of stimuli of different frequencies. To facilitate this equalization, they have constructed a compensatory filter having a response characteristic, the inverse of that of the skin, within about 2 or 3 dB. Work is proceeding on construction of a special direct-coupled amplification and switching circuitry required for the accurate reproduction of low frequency pulse and square wave stimuli waveforms. The equipment used to date is of the standard AC coupled variety and causes some distortion at the lowest frequencies of interest in tactile studies. Psychophysical testing,using the new facilities, has begun and data is now being used on the frequency discrimination capabilities of the skin for sinusoid and pulse trains. For sinusoidal stimulation, preliminary results on three subjects are consistent with data reported in the literature. A significant improvement in frequency discrimination at low frequencies (below 50 Hz) was found when a train of pulses at about 1.5 milliseconds wide was used as the stimulus instead of the sinusoid. Above about 100 Hz, the data for the pulse trains become similar to that for sinusoidal stimulation. Preliminary measurements of threshold as a function of frequency verify that threshold varies very little for pulse trains as compared to sinusoidal stimulation. These findings support the concept of using variable frequency pulse trains for encoding information in a tactile vocoder instead of fixed frequency sinusoids.

In an attempt to elucidate the role of the sense of touch in communication, a group of investigators reported the systematic investigation of the cutaneous "rabbit" or saltatory induction effect which is cutaneous illusion. They found it is generally possible to produce saltatory induction if two or more vibrotactile transient stimuli (i.e., short taps to the skin) are presented to one body locus, and immediately thereafter one or more taps are presented to a second locus. The observer will report, if the times between the stimuli are from 50-100 msec., that the taps are localized between the sites of stimulation, quite as though a tiny rabbit had hopped from the first site to the second. The investigators confirmed preliminary findings that increasing the number of loci and their separation served to spread the effect over a wider area. A linear arrangement of contractors was unnecessary, contours of a complex sort, e.g., letters, simple shapes, etc., could be generated if each locus was excited twice during a progressive pass through the points. A simple line can be produced if only two points are excited, the first four times and the second only once, provided the timing is within the limits stated above.

When the second locus is moved too far from the first or placed across the body midline, or in certain other relations to the first, an "incomplete rabbit" may appear. This is not to be confused with the reduced rabbit. The incomplete rabbit is a hopping that begins well, then disappears at some point in the interstimulus space, leaving a hole between loci where perceptible hops should be. If the longitudinal distance on the arm exceeds about 35 cm or if the loci are across the midline of forehead, chest or abdomen, or laterally on the forearm separated by 5 cm, an incomplete rabbit appears. Dermatomal boundaries might be involved in this zonal barrier effect. Comparison of these barriers contours with the innervation boundaries described by neurological research is proceeding.

For the full rabbit, the situation in which three or more taps are given at the first locus and one or more at the second in interstimulus interval, (isi) for hopping may vary from about 200 msec down to the limit of their present system 20 msec, with the optimal hopping of about 50 to 60 msec. The latter values induce well distributed, clearly perceptible sets of loci on the integument between contractors. The "reduced rabbit" is generated by presenting a locator tap to locus 1 followed in 0.8 sec by a second tap which is followed by a third tap; a variable time delay at locus 2.

The question of the physical basis for saltation was dealt with and answered in the negative. The phenomenon is surely central and not dependent on peripheral tissue mechanics or receptor characteristics. Changes in contractor coupling to the skin, displacement directions, presence of heat or cold, prove to have little or no effect on saltation. Topical anesthesia of a strip of skin between contactor did not affect the localization of taps despite the fact that the skin of the treated area was less sensitive to direct tactile stimuli. The taps were felt as clear as ever, but deeper in the skin than usual.

Auditory saltatory induction is being investigated, using transient clicks from different points in space around an horizontal plane. Visual saltatory induction using fiberoptics in a dark room and a radius of curvature mounted at eye level is also being investigated.

ANNUAL REPORT
July 1, 1974 through June 30, 1975
Extramural Programs
Special Programs Branch
National Institute of Neurological and Communicative Disorders and Stroke

Fiscal year 1975 activities in the Special Programs Branch were generally routine in so far as Research Program Projects, Clinical Research Centers, Outpatient Clinical Research Units, and Stroke Acute Care Research Units are concerned and the significant research findings resulting from the support of research funded through these programs appears in other parts of this report. Concerning training activities, the year had some unusual aspects and this section of the report is devoted exclusively to the Institute's training programs and activities.

In February 1973, we began phasing-out support for all training programs in effect at that time with the phase-out being completed when the Institute had honored its commitments by providing all periods of recommended support. The following programs were involved: "Old" Training Grant, Individual Postdoctoral Fellowship, and NINCDS Special Traineeship. This phase-out plan has continued according to schedule.

FY '75 funds were used to provide phase-out support for 152 training programs, 39 fewer programs than were supported a year earlier. (See Appendix B for the distribution of these programs among training areas.) As in the previous year, programs receiving their final year of recommended support received two-year budget periods. It was thought that the procedure would give program directors more flexibility in the use of NINCDS funds and thus provide for a more orderly termination of NINCDS support for training programs.

One change in the "Old" Training Grant Program was an NIH decision to increase predoctoral stipends to a single $3,900 level regardless of the year of predoctoral training and the number of dependents. Program directors who elected to use the new predoctoral stipend level were not given additional funds.

Only seven Postdoctoral Fellows were supported from FY '75 funds, a reduction of 11 from the number supported the previous year and ten NINCDS Special Trainees were supported from FY '75 funds, a reduction of eight from the number supported a year earlier.

Under the phase-out guidelines, in the spring of 1973, it was anticipated that the Institute would not make additional NINCDS Teacher-Investigator Awards or Research Career Development Awards. However, the identity of these programs was changed from training to research and likewise the funding changed from training to research and the programs were reactivated. Since applications had not been received for well over a year, as was anticipated, a backlog of institutions wanting to submit applications on behalf of T-I or RCDA candidates developed. This resulted in the submission of a larger number of applications than we had previously experienced. Of 112 new RCDA applications submitted, 20 were funded and of 24 new T-I applications submitted, seven were funded. This is compatible with the Institute's long range plan of making approximately 25 of these awards annually and of having approximately 125 individuals supported by these programs at any one time. (See appendices C and D for the distribution of RCDA and T-I applications, both new and continuation, among training areas.)

Concerning the 112 new RCDA applications that were submitted, 64 (57%) were recommended for approval and 48 (43%) were recommended for disapproval. This is mentioned because 13 of the disapproved applications were submitted on behalf of candiates whom the reviewing bodies considered over-qualified.

Simultaneous with the phase-out of the training programs, NIH initiated two new training activities which were authorized by P.L. 93-348, the National Research Act, which became effective July 12, 1974. This legislation authorized the NIH to make Institutional National Research Service Awards and Individual National Research Service Awards and these new programs resulted in the phasing-out of the "Weinberger" Individual Research Fellowships, a program in which the Institute had just made a number of awards to individuals. We had earlier made a decision not to make institutional awards under the "Weinberger" authority. To honor the Institute's commitment to these "Weinberger" individual Awardees, it is estimated that 18 continuation awards will be made out of FY '75 funds. An appreciably larger number of individuals will be in training under this program but all applicants initially recommended for two years of support were given two-year awards thus making it necessary to make only the $3,000 Institutional Allowances for these specific fellows. The only "Weinberger" Awardees who received stipend support from FY '75 funds were those who were initially recommended for three years of support. (See appendix E for the distribution of all fellows among training areas.)

At the time the National Research Act became law, a number of program directors were in the process of preparing institutional applications for submission under the "Weinberger" authority. When these were received, the program directors were given the option of withdrawing their applications or having them reviewed under the policies and guidelines governing the programs authorized under the authority of P.L. 93-348. Of the applications reviewed by the DRG study sections and the NANCDS Council in March, 16 were recommended for approval and 11 were recommended for disapproval. Of those recommended for approval, the following six were funded for the support of postdoctoral trainees only:

Grant Number	Program Director	Institution	Training Area
1 T01 NS			
7010-01	Lloyd Beidler	Florida State Univ.	Sensory Physiology
7012-01	Robert Cutler	Stanford Univ.	Developmental Neurology
7011-01	Norman Geschwind	Harvard Univ.	Sensory Physiology
7026-01	Joseph Hind	Univ. of Wisconsin	Sensory Physiology
7000-01	Richard Johnson	Johns Hopkins Univ.	Neurovirology
7027-01	Arthur Prensky	Washington Univ.	Developmental Neurology

Twenty-six Institutional National Research Service Award applications will be reviewed at the June meeting of the National Advisory Neurological and Communicative Disorders and Stroke Council. Of these, twenty-one have been recommended for approval by the initial reviewing bodies. It is anticipated that some of these programs will be supported from FY '75 funds but the specific number is not known.

One feature of the Institutional National Research Service Award Program is that awards can be made in only five specified training areas for which the Institute prepared a special justification and which were approved. These are

Developmental Neurology, including spinal cord regeneration
Neurovirology
Neuroimmunology
Sensory Physiology and Biophysics
Minority training program in the neurosciences

As a result of our experiences with these five areas, it became obvious that additional training areas were desirable and we were later approved for making Institutional National Research Service Awards in two additional areas, (1) neuropathology and/or otopathology and (2) neurobiology.

The Institutional National Research Service Award Program is different from the "Old" Training Grant Program in at least three significant respects. (1) Ultimately, training awards will be made only in specified areas which are identified as needing additional personnel. This aspect of the Program is specified in the legislation and a study to determine specific areas in which support can be provided will be carried out by the National Research Council of the National Academy of Sciences. (2) The legislation includes a "pay-back" provision which requires trainees who receive direct support under this legislative authority to accept academic or research positions following the completion of their training or to reimburse the government for the cost of their training. (3) A relationship exists between the amount that can be requested for trainee expenses and the amount provided for environmental support. The latter can constitute not more than 25% of the direct trainee costs (predoctoral and postdoctoral trainee stipends, tuition and trainee travel for predoctoral trainees, a $1,000 Institutional Allowance for each postdoctoral trainee, and the overhead [8%] on the allowable trainee expenses).

The Institute was authorized to make Individual National Research Service Awards in 14 areas. Following the publication of the policies governing the program in the Federal Register, the Institute made 77 postdoctoral awards. The following is the number of individual awards according to training area:

Area	No. of Awards
Audiology	0
Clinical Investigation	0
Developmental Neurology	6
Neuroanatomy	6
Neurobiology	10
Neurochemistry	17
Neuroimmunology	1
Neuropathology and/or Otopathology	0
Neuropharmacology	13
Neurophysiology	16
Neuroradiobiology	0
Neurovirology	0
Sensory Physiology and Biophysics	8
Speech Pathology	0

These areas were selected because of the need to train additional personnel to conduct research in them and because it was thought that a number of training programs exist which already have the personnel, space, and equipment required to provide postdoctoral research training.

Additional Individual National Research Service Awards will be funded out of FY '75 funds but the specific number has not been determined. These additional awardees will be selected from approximately 201 applications that will be reviewed during the June meeting of the National Advisory Neurological and Communicative Disorders and Stroke Council.

Manpower Evaluation Studies

In the fall of 1972, the Institute awarded four contracts to conduct manpower evaluation studies in neurology, neurosurgery, otolaryngology, and speech pathology and audiology. These were awarded to the American Neurological Association, the American Association of Neurological Surgeons, the American Council on Otolaryngology, and the American Speech and Hearing Association respectively. Only one of these contractors, the American Speech and Hearing Association, has submitted a final report and the Association has published a summary of their findings in its April 1975 issue of *Asha*.

In June 1973, the Institute awarded a contract to the National Research Council of the National Academy of Sciences to assess manpower needs in the basic sciences related to neurology and the communicative areas and it is anticipated that their study will also require about two years to complete.

APPENDICES

Following are four appendices which show how support for the Institute's training programs is divided among the various training areas.

Appendix B shows how support for "Old" Training Grants, currently in a phase-out status, is divided among the training areas.

Appendix C shows how both new and continuation RCDA Awardees are divided among the training areas.

Appendix D shows how both new and continuation Teacher-Investigator Awardees are divided among the training areas.

Appendix E shows how the "Weinberger" Individual Research Fellowships are divided among the training areas. These are continuation awards only since the program is in a phase-out status.

APPENDIX B

Distribution by Scientific Fields, of Training Grants Awarded in FY 1975

FIELD	NUMBER	AMOUNT
Audiology	5	$ 360,000
Child Neurology	11	697,100
Communicative Disorders	3	258,600
Neuroanatomy	2	98,700
Neurobiology	4	235,900
Neurochemistry	2	107,200
Neurological Sciences	2	86,800
Neurology	49	3,004,700
Neuropathology	8	383,600
Neuropharmacology	3	214,700
Neurophysiology	9	561,100
Neuroradiology	7	369,100
Neurosurgery	14	768,600
Neurovirology	1	42,100
Otolaryngology	29	2,107,600
Sensory Physiology	2	95,700
Speech Pathology	1	36,500
TOTAL	152	$ 9,428,000

May 1975

APPENDIX A

RESEARCH GRANTS AWARDED IN FY 1975 BY DISORDER CATEGORY (Cont'd.)

(DOLLARS in THOUSANDS)

DISORDER CATEGORY	NO.	AMOUNT	% of $
2. SENSORY AND PERCEPTUAL DISORDERS			
A. Disorders of Hearing or Equilibrium	120	9,436	10.9
B. Disorders of Speech or Other Higher CNS Functions	34	2,294	2.6
C. Disorders of Other Senses	151	8,501	9.8
TOTAL - SENSORY & PERCEPTUAL DISORDERS	305	20,231	23.3
3. MULT-CATEGORICAL	18	5,403	6.2

APPENDIX A

RESEARCH GRANTS AWARDED IN FY 1975 BY DISORDER CATEGORY

(DOLLARS in THOUSANDS)

DISORDER CATEGORY	NO.	AMOUNT	% OF $
TOTAL ALL DISORDERS	1198	86,643	100.0
1. NEUROLOGICAL DISORDERS			
A. Neurological Disorders of Early Life	168	9,117	10.5
B. Neurological Disorders of Aging	48	3,639	4.2
C. Cerebrovascular Disorders	56	7,378	8.6
D. Convulsive or Related Paroxysmal Disorders	46	3,757	4.3
E. Demyelinating or Sclerosing Disorders	42	4,881	5.6
F. Muscular or Neuromuscular	131	6,729	7.8
G. Infectious Diseases	19	1,132	1.3
H. Trauma or Injury	78	10,590	12.2
J. Tumors or Nervous System	14	637	0.7
M. Neuroendocrine Studies	80	3,960	4.6
N. Neural Aspects of Learning or Behavior	49	2,480	2.9
P. Nervous System Studies - Normal Function	144	6,719	7.8
TOTAL- NEUROLOGICAL DISORDERS	875	61,009	70.5

APPENDIX C

Distribution, by Scientific Fields, of Fellowships Awarded in FY 1975

Field	RESEARCH CAREER AWARDS No.	RESEARCH CAREER AWARDS Amount	RESEARCH CAREER DEVELOPMENT AWARDS No.	RESEARCH CAREER DEVELOPMENT AWARDS Amount
Audiology	1	30,000		
Biochemistry			1	17,600
Biophysics				
Cerebrovascular	1	31,000		
Communicative Disorders			1	20,270
Epidemiology			1	16,350
Immunology			2	36,000
Neuroanatomy			4	86,000
Neurobiology			12	244,600
Neurochemistry			13	235,350
Neuroendocrinology	1	30,000	3	65,600
Neurological Sci., Multi.			1	25,000
Neurology	1	32,000	1	25,000
Neuropathology			3	71,780
Neuropharmacology	2	62,000	4	87,000
Neurophysiology			14	290,200
Neuroradiology			1	20,000
Neurovirology			4	66,500
Otolaryngology	1	33,000		
Physiological Psychology	1	32,000	2	32,000
Sensory Physiology			3	68,750
TOTAL	8	250,000	70	1,480,000

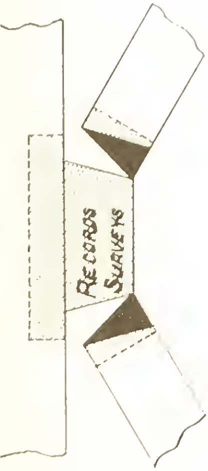
May 1975

HOW TO USE
THESE SEPARATORS

Use one page for each separation.

Select appropriate tab, add further identification if desired, and cover it with scotch tape.

Cut off and discard all tabs except the one covered by tape.



TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

ANNUAL REPORT

July 1, 1974, through June 30, 1975
Extramural Programs
Grants Management Section
National Institute of Neurological and Communicative Disorders and Stroke

The Institute's Extramural Grant Funds accounting reflects amounts in the four budget activity areas:

1. Communicative Disorders
2. Neurological Disorders
3. Stroke, Nervous System Trauma
4. Fundamental Neurosciences

Within each budget activity area research funds are classified under regular, program project or specialized program project centers. The fiscal data in the grant budget is reviewed once a month with Office of Financial Management. Considerable requests for revisions have resulted this past fiscal year due to a greater number of Common Account Numbers and the need for the sub-account groupings (type 1, 2, 3, 5 funds) within these numbers.

Equipment Accountability Disposition forms were still being submitted by the grantees but on a reduced scale as its requirement terminated as of July 1, 1974. The backlog of Terminated Grant Files has been reduced. The Clerk-Typist position has been filled on a less than full time basis. Until the Clerk-Typist was recruited, our Grants Clerk had to put in considerable overtime in attempting to stay abreast of the office's workload. By reducing the backlog, we have found that the Terminated Grant files closed within the past two fiscal years have resulted in less requests for necessary reports.

The Grants Clerk's work scope will gradually be increased so that it becomes a ladder position to a Grants Technical Assistant. Increased funding of our research grants program in FY'75's last quarter has resulted in a greater number of grants undergoing review for budget revision and managerial review with the program staff.

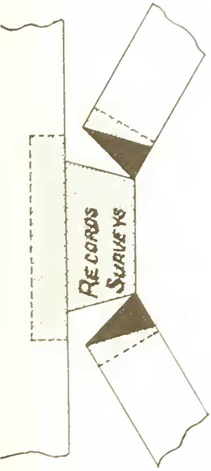
The new National Research Service Award Guidelines for Individual and Institutional Awards are undergoing policy assimilation by our management staff. Budget requests for Institutional Awards require considerable review due to the 25% total award allowance for non-trainee expenses.

HOW TO USE
THESE SEPARATORS

Use one page for each separation.

Select appropriate tab, add further identification if desired, and cover it with scotch tape.

Cut off and discard all tabs except the one covered by tape.



TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

ANNUAL REPORT

July 1, 1974 through June 30, 1975

Extramural Programs
Data Analysis and Reports

National Institute of Neurological and Communicative
Disorders and Stroke

The Data Analysis and Reports Section is fully operational with its computerized information system which operates, for greater accuracy and flexibility, in parallel with the manual system. The data base provides generally comparable data for the FY 1965 to FY 1975 time span on all research and training grants, fellowship awards and indirect trainee appointments funded by the NINCDS Extramural Programs. Considerable update and modifications of existing programs along with the development of some new programs have been accompanied by a documentation of all software. New procedures designed by the programmer staff and implemented by the input unit have eliminated any present need for a computer technician. They have improved editing efficiency by eliminating the need for keypunching.

The Section, in addition to regularly published data books and fiscal year end summary books, has prepared special reports for the Institute's three regularly scheduled Advisory Council meetings in FY 1975. These reports compared the extramural grant trends over the past six years by type of grant and program area (March Council) and then analyzed the research grants reviewed, approved, and awarded in FY 1972-1975 by program area (June Council). Several special studies were prepared during the year--Otolaryngology Research Supported by the NIH, FY 1974 and NINCDS Research as It Relates to the Recommendations of the National Advisory Commission on Multiple Sclerosis. The NINCDS Targeted Research Grant List was revised to provide more detail. The Section also responded to special requests (typically 3 to 5 or more per week) for the Office of the Director, usually processed through the Budget Office, for the Program Planning and Evaluation Office, or for the Information Office.

The staffing problems of FY 1974 have largely vanished. In addition to the permanent staff we have a part-time STRIDE employee. No unusual delays or data management problems are anticipated.

7435

[illegible]
[illegible]
[illegible]
[illegible]

LIBRARY
NIH
Amazing Research.
Amazing Help.

http://nihlibrary.nih.gov

10 Center Drive
Bethesda, MD 20892-1150
301-496-1080

DATE DUE

GAYLORD
PRINTED IN U.S.A.

NIH LIBRARY
4 0129 1592

WERT
BOOKBINDING
MIDDLETOWN PA
NOVEMBER 75
We're Quality Bound

NIH LIBRARY
3 1496 00186 8200